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THE CONTROL OF DANDELIONS IN LAWNS[1]

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INTRODUCTION

The common dandelion (*Taraxacum officinale* Weber) is the most troublesome weed of lawns in the cooler portions of the United States. The flattened leaf rosette and contractile perennial taproot adapt the plant to growth in closely mowed or pastured grasslands, while its ability to withstand drought or unfavorable conditions when once established, and to regenerate new crown buds, even when cut at depths of 8 to 10 cm below the surface, contribute to its permanence.

The growth of the dandelion in sod, and particularly in lawns, prevents the use of cultivation or the usual herbicides as control measures. Deep hand cutting and iron sulphate sprays have been most commonly used, but neither method is effective unless repeated several times at frequent intervals and under favorable conditions.

Work on measures for the control of dandelions was initiated at Ames, Iowa, by Dr. I. E. Melhus and has been pursued intermittently for the last 9 years. The results of some of the earlier experiments have been published by Melhus (*18*)[2] and Arnold (*3*).

The work on dandelion control reported here may be divided into three sections: (1) that concerned with the biology and particularly with the food reserves of the plant; (2) the use of distillates as differential herbicides for the control of dandelions in bluegrass sod; and (3) the ecological factors concerned in the establishment and maintenance of dandelion-free lawns.

THE DANDELION ROOT

Aside from mechanical removal, the eradication of dandelions depends either upon killing the fleshy taproot or so weakening it as to prevent regeneration after cutting or spraying treatments. The taproot system of the plant extends 3 or more feet into the ground, and much of the root system of the older plants extends below the level of the grass roots. This deep rooting is an important factor in the ability of the plant to compete with bluegrass or other sods.

The upper portions of the taproot, which may attain a diameter of 2 cm, contain the abundant xylem and phloem parenchyma characteristic of storage roots. Figure 1, *A* and *B*, shows this abundance of parenchyma, particularly in the phloem. Figure 1, *B*, shows also two features characteristic of older roots; namely, (1) the cutting off and sloughing of the older phloem, and (2) the tendency toward

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[2] Reference is made by number (italic) to Literature Cited, p. 867.

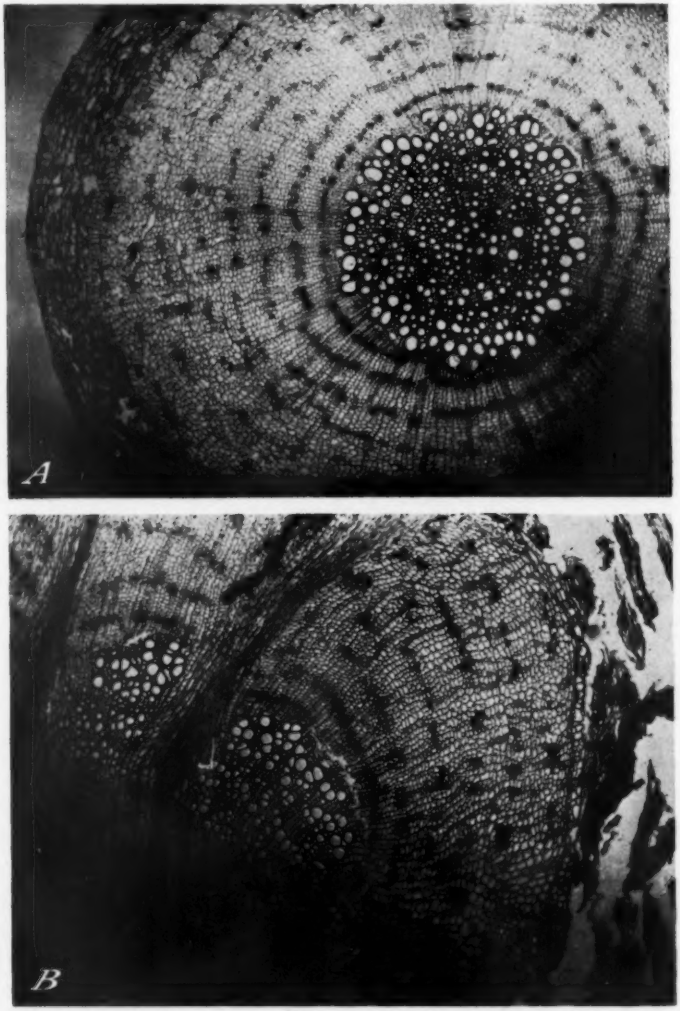

FIGURE 1.—Cross sections of dandelion root: *A*, Young root showing abundant parenchyma and scattered groups of sieve tubes in the phloem; *B*, older root showing sloughing of primary phloem and formation of interxylem phloem strands.

proliferation of the xylem parenchyma with the separation of xylem strands, the development of internal phloem, and frequently the separation of the older root into several strands, separate at the top with separate crowns but joined at the base.

RESERVES OF THE ROOT

Perennial composites frequently form inulin or levulins as storage polysaccharides. The artichoke (*Helianthus tuberosus*) and dahlia

(*Dahlia* spp.) are familiar examples. Preliminary tests showed that the dandelion root contains levulins, substances comparable to the dextrins but more easily hydrolyzed and yielding fructose instead of glucose. Levulins are distinguished from inulin by their greater solubility in cold water.

A 5-g sample of root residue, which had been killed and thoroughly extracted with 80-percent alcohol to remove sugars, dried, and ground to 200 mesh in a ball mill, was extracted 10 times with cold water. Each extraction was hydrolyzed separately with 1+100 [3] HCl at 70° C. for 35 minutes, and the reducing value of one-fifth aliquots determined by the Munson-Walker-Bertrand method as given by Loomis and Shull (*17*). The residue from the cold water extraction was then extracted 10 times with boiling water by suspending in hot water in centrifuge tubes immersed in a boiling water bath, and stirring intermittently for 30 minutes. The residue was centrifuged out and each extraction hydrolyzed separately as above and tested for reducing substances.

TABLE 1.—*Reducing substances yielded by successive hot- and cold-water extractions of a sugar-free dandelion root powder*

Extraction No.	Cold-Water extractions		Hot-water extractions		Extraction No.	Cold-water extractions		Hot-water extractions	
	Reducing substances	Percentage of total	Reducing substances	Percentage of total		Reducing substances	Percentage of total	Reducing substances	Percentage of total
	Milligram	*Percent*	*Milligram*	*Percent*		*Milligram*	*Percent*	*Milligram*	*Percent*
1	50.2	73.4	0.9	30	7	0.3	0.4	0.3	10
2	12.1	17.7	.3	10	8	.3	.4	.3	10
3	3.1	4.6	.3	10	9	.5	.7	.5	17
4	1.0	1.6	.4	13	10	.0	.0	.0	0
5	.7	1.0	.0	0					
6	.2	.2	.0	0	Total	68.4	100.0	3.0	100

The results of the two tests, given in table 1, show that only cold-water-soluble polysaccharide reserves were present. Inulin and portions of any starch present would have been extracted by the hot water, but the data indicate that only traces of material, such as would have been obtained from slow hydrolysis of hemicelluloses, were found in the hot-water extract. The absence of starch was shown by the iodine test also, and it is inferred that starch and inulin are not normal storage forms in dandelion.

The cold-water extract from 5 g of powder was made to 1 liter and 50-ml aliquots used for hydrolysis tests as indicators of the contained materials. Fifty milliliters of this extract, cleared with neutral lead acetate and hydrolyzed under inulin hydrolysis conditions (1+100 HCl at 70° C. for 35 minutes (*13*)), gave a reducing value of 17.6 mg when calculated as invert sugar. The uncleared solution gave a value of 19.4 mg which is higher than the value for the cleared solution by seven times the error of determination. The loss could have been due either to occlusion of levulins with the lead precipitate or to the presence of a levulin gum containing an acid radical and thus forming a lead salt. When the hydrolysis time at 70° C. with 1+100 HCl

[3] One part of concentrated c. p. HCl and 100 parts of water by volume. Approximately 0.43 percent hydrogen chloride by weight.

was increased by intervals from 0 to 120 minutes, the invert sugar yields from 50-ml aliquots of the solution varied as indicated below.

Period of hydrolysis at 70° C. (minutes)	*Reducing substances from 50 ml extract as invert sugar (milligrams)*
0	1.4
10	18.7
20	19.2
30	19.6
40	19.7
60	20.3
120	21.3

Levulin hydrolysis should be completed in 35 minutes; it will be noticed that it was very nearly completed in 10 minutes, but that there was a slight increase in reducing value with continued heating in spite of the slow destruction of levulose by the heating. The continued rise with time suggested a mixture of dextrin and levulin, and hydrolysis with 1+20 HCl at 120° C. for 30 minutes gave a reducing sugar value of 28.7 mg.

The material was tested further for the presence of dextrins by a series of hydrolyses, with another sample of extract, in which acid concentration, heating time, and heating temperature were varied independently. The results, given in table 2, indicate the presence of a mixture of levulin and dextrin in approximately equal quantities. The reducing value of this extract after levulin hydrolysis (1+100 HCl at 70° C. for 35 minutes) was 11.6 mg invert sugar in 50 ml of extract; after dextrin hydrolysis (1+20 HCl at 120° for 60 minutes), it was 19.8 mg; and after a shorter hydrolysis at the higher temperature, to hydrolyze most of the dextrin with a minimum destruction of levulose, the value was 21.5 mg. Heating in 1+40 HCl for 120 minutes did not give a significantly different value. Shorter heating with the weaker acid gave low values, suggesting an incomplete hydrolysis of dextrins.

TABLE 2.—*Hydrolysis of cold-water extract of dandelion residue with 1+20 and 1+40 HCl at 70° and 120° C.*

Heating time (minutes)	Temperature	Reducing sugar[1] per 50 ml when hydrolyzed with indicated concentration of acid		Heating time (minutes)	Temperature	Reducing sugar[1] per 50 ml when hydrolyzed with indicated concentration of acid	
		1+20 HCl	1+40 HCl			1+20 HCl	1+40 HCl
	°C.	*Milligrams*	*Milligrams*		°C.	*Milligrams*	*Milligrams*
35	70	12.3	12.2	60	120	19.8	17.1
30	120	21.5	16.7	120	120	18.5	22.3

[1] 1+100 HCl at 70° C. for 35 minutes gave an invert sugar value of 11.6 mg per 50 ml of extract.

SEASONAL FLUCTUATIONS IN THE RESERVES OF THE ROOT

The effectiveness of any method of dandelion control which kills or removes only the upper portions of the plant will depend directly upon the food reserve level of the roots at the time of treatment. The effectiveness of other types of treatment also may be correlated with reserve level.

Samples were taken at approximately monthly intervals during the summer of 1935. The season was normal, and dandelions were

dominant in the area from which the samples were dug. The washed roots were sliced into boiling alcohol and extracted and the several fractions determined with methods given by Loomis and Shull (*17*). The reserve polysaccharide determination was simplified by hydrolyzing with 1+20 HCl for 30 minutes at 120° C. Results so obtained are approximately 10 percent low because of the destruction of levulose at the high temperature. The analytical data are summarized in table 3. The low point in total nitrogen came at the time of fruiting, but the low point in carbohydrate reserves was apparently reached during early vegetative growth. Instead of a drop during flowering, the polysaccharides, which were the dominant carbohydrate reserve, increased from the first sampling on May 1 to a high during a short dry period in July and remained high except for a dip with new growth following late July rains. The carbohydrate analyses suggest that treatments, such as cutting or iron sprays, the effectiveness of which depends upon low reserves, should be made early in the season.

TABLE 3.—*Carbohydrate and nitrogen content on fresh-weight basis of dandelion roots at different times of the year*

Date of sampling	Reducing sugar	Sucrose	Polysaccharides	Total carbohydrate	Soluble nitrogen	Protein nitrogen	Total nitrogen
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
May 1 (budding)	0.25	0.77	1.25	2.27	0.12	0.12	0.24
May 20 (bloom)	.13	.69	2.66	3.48	.04	.23	.27
June 1 (fruiting)	.33	1.09	3.38	4.80	.04	.11	.15
July 8	.56	1.08	5.53	7.17	.04	.13	.17
Aug. 6	.57	.85	2.59	4.01	.06	.17	.23
Sept. 10	.36	.74	3.68	4.78	.10	.15	.25
Oct. 16	.32	1.83	3.04	5.19	.14	.25	.39
Nov. 8	.25	1.51	3.90	5.66	.27	.20	.47

HERBICIDES FOR THE CONTROL OF DANDELION

Iron sulphate (ferrous) was widely advocated for weed control by Bolley (*5*) at the beginning of the century. He stated that dandelion plants were prevented from seeding but not killed by four sprays. Adams (*2*) and Moore and Stone (*19*) likewise recommended iron sulphate, but apparently they took their records shortly after spraying and before the plants had resprouted from the roots. Arthur (*4*), French (*7*), Fyles (*8*), and Olive (*21*) report that the leaves were quickly blackened and killed, but that the plants resprouted from the roots even after repeated treatments. French sprayed 12 times in 2 years without killing the stronger dandelion plants. On the other hand, Munn (*20*) and Longyear (*15*) reported satisfactory control with three or four sprays properly applied. In the present writer's work, single sprays in connection with heavy fertilization and under favorable conditions have given a 40-percent control, and three sprays under less favorable conditions have given no control.

Other herbicides recommended by various authors include sulphuric acid (*1*), ammonium thiocyanate,[4] furfural emulsions (*3*), ammonium sulphate (*6*), gasoline, and kerosene. Of these, the first three burn or kill the grass when applied at rates which are effective against

[4] HARVEY, R. B. AMMONIUM SULFOCYANATE AS A WEED SPRAY. Minn. Univ., Div. Plant Physiol. Mimeographed Pub. 1930.

dandelions. Gasoline burns the grass and is too volatile to be used as a spray in warm weather. Kerosene was used by Longyear (*15*) and Olive (*21*) for individual plant applications made with an oilcan, but its use as a differential spray was first reported by Loomis and Noecker (*16*).

Kerosene and other distillates have been used on the Iowa State College campus on some 200 experimental plots and on two 5-acre and one 30-acre area. The results have varied from a 50-percent mortality of dandelions and serious injury to the grass to a 100-percent mortality of dandelions from a single application and no permanent injury to bluegrass or white clover sods. Kerosene specifications and weather at and after the time of spraying appear to be the factors that determine the success or failure of the treatment.

SPECIFICATIONS OF A KEROSENE FOR LAWN SPRAYING

The boiling-point range for commercial kerosenes is commonly 180° to 250° C. Perfection kerosene, a commercial product with a boiling-point range of 185° to 240°, was compared with distillation cuts from a specially purified lot of naphtha, prepared and supplied by an oil refinery in Okmulgee, Okla. This latter product was treated to reduce the percentage of unsaturated hydrocarbons, and then fractioned into low, medium, and high boiling-point-range samples. A sample of acid-washed Red Crown gasoline having a boiling-point range of 40° to 180° was included in the test. The results of the experiment are given in table 4. They indicate that the medium boiling-point fractions were most satisfactory for the conditions of the spraying, a cool day in mid-October. The distillates having the higher boiling points left an oily residue on the sod and seriously injured or nearly killed the grass as well as the dandelions. Probably both residue and grass injury with these fractions would have been less noticeable had the spraying been done in warmer weather.

TABLE 4.—*Percentage of dandelions killed and of bluegrass injured with various hydrocarbon sprays having different boiling points, applied during cool weather in mid-October*

[Data are averages of those from three 100-square-foot plots]

Boiling-point range (° C.)	Dandelions killed	Bluegrass injury	Boiling-point range (° C.)	Dandelions killed	Bluegrass injury
	Percent	*Percent*		*Percent*	*Percent*
40–180 [1]	95	40	245–300 [3]	100	60
180–240 [2]	98	20	300–325 [3]	99	99
190–245 [3]	98	30			

[1] Acid-washed gasoline—Red Crown.
[2] Acid-washed Perfection kerosene.
[3] 3 fractions of refinery purified distillate.

Gasoline sprays in warm weather have resulted in a quick burn of both grass and dandelions, but few if any of the dandelion plants have failed to recover. Kerosene may give a poor kill, apparently because of its volatility, when used in hot weather, and it seems probable that a dandelion spray for general conditions could include distillates up to 275° C. when mixed with the lighter kerosene. Kerosene (180°–250°), however, can be used alone in any but the hottest

weather. The inclusion of fractions with a boiling-point range below 180° would be permissible in cool weather but would add to the cost of the treatment.

The unsaturated hydrocarbon content of distillates has been shown (*22*) to be a major factor in their toxicity to foliage, and the writer has found that oils containing considerable percentages of unsaturated compounds destroy bluegrass sods as well as dandelion plants. A number of distillates of the kerosene boiling-point range of 180° to 250° C. and different percentages of unsaturated hydrocarbons have been used in various tests. Perfection kerosene is described by the refiners as a straight-run distillate, that is, one obtained from the first distillation of the crude oil before cracking treatments are used, and as containing from 2.5 to 4 percent of unsaturated hydrocarbons. Recycle gas oil, also known as tractor distillate, is the same boiling-point cut on the cracked residue from the first distillation. The breaking of the long-chain, high-boiling-point hydrocarbons in cracking leaves many unsaturated compounds, and recycle gas oil may run 20 percent or more unsaturated. Distillates prepared for such products as fly-spray bases are washed with concentrated sulphuric acid or equivalent treatments which reduce the percentage of unsaturated compounds to approximately 1 percent. Whenever these three preparations have been compared it has been found, as shown in table 5, that the gas oil or tractor distillate destroys bluegrass as well as dandelions. Oil with 3 to 4 percent of unsaturated compounds appears to be safe under favorable conditions. Commercially purified oils, or kerosenes washed with concentrated sulphuric acid, have, in three trials, been somewhat less effective in eradicating dandelions than the straight-run kerosene. Injury to the grass by late fall (November 1) spraying was not prevented by washing kerosene with acid. More exact determinations of the tolerance of bluegrass to unsaturated hydrocarbons are needed, but present findings indicate that an unsaturated content of not more than 4 percent should be specified.

TABLE 5.—*The relation of unsaturated hydrocarbon content of distillate sprays to dandelion control and grass injury*

[Data are averages from three replicated plots]

Hydrocarbon used	Percent unsaturated [1]	Mature dandelions per 100 square feet	Dandelions killed	Grass injury	Seedling dandelions per 100 square feet [2]
		Number	*Percent*	*Percent*	*Number*
Check		657	0	0	23
Perfection kerosene	3	10	98.5	[3] 27	80
Acid-washed kerosene	1	10	98.5	30	80
Gas oil	25	3	99.5	100	370
Standard tractor distillate	18	3	99.5	85	120
Acid-washed distillate	3-5	3	99.5	40	30

[1] Average figures for type of material used.
[2] Seedling counts in May after spraying in October; they represent new invasion.
[3] Injury more severe than normal because sprays were applied in late October.

CONTROL OF DANDELIONS WITH KEROSENE

Methods of Application

Two methods of applying kerosene may be used. Individual plants may be sprayed (fig. 2, *A*) when scattered plants are to be eradicated,

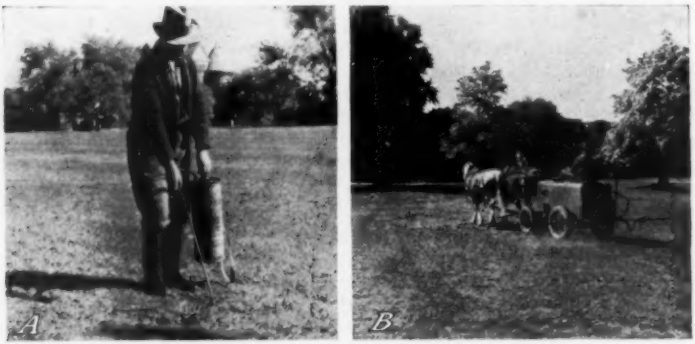

FIGURE 2.—Applying kerosene for dandelion control: *A*, Method for scattered plants or small areas; *B*, spraying large areas.

or broadcast sprays (fig. 2, *B*) may be used for larger areas and heavier infestations. Individual plant applications can be used over a wider range of conditions and are safer for lawn use. Just enough kerosene to wet the crown and most of the leaves of the plant should be applied as a fine spray. If a lawn is treated in part by spotting and in part by spraying, an irregular growth for 2 or 3 weeks will result from the checking of the growth of the grass in the sprayed areas.

Power sprayers, such as the one illustrated in figure 2, *B*, should be adjusted to cover the surface uniformly at a rate of 200 gallons an acre. Kerosene atomizes readily and a high pressure is not required in its application. Although such a machine has not been used, good results would be expected with a traction-driven potato sprayer. If the spray is applied in the fall, as recommended, incidental spraying of deciduous shrubbery will do no harm. Evergreens should not be sprayed.

Weather Factors

Kerosene may be applied to wet grass or during a rain without appreciable loss of effectiveness. In fact, sprays so applied, because of less loss from evaporation, may be more effective than those applied in clear weather. The opposite relationship holds for ferrous sulphate sprays which are washed from the leaves by rain.

Temperature and sunshine are important factors in the effectiveness of kerosene sprays for dandelions. One comparison of their effect was made on July 21, 1935. One of two plots was sprayed at 10:30 a. m. in bright sunlight, temperature 35° C.; the second plot was sprayed after sundown of the same day, temperature 22°. On October 1 the percentage mortality of dandelions was 20 and 85, whereas grass injury was 80 and 0 percent. Additional data are given in table 6.

TABLE 6.—*Relation of weather factors to the effectiveness of kerosene sprays, 1935*

[Dandelion counts on May 5, 1936; all data are average of three replications]

Spraying treatment [1]	Mature dandelions per 100 square feet	Dandelions killed	Grass cover	Seedling dandelions per 100 square feet
	Number	*Percent*	*Percent*	*Number*
Check	699		34	51
Sept. 11, 26° C., sun, 5 p. m.	213	67	43	200
Sept. 25, 22°, cloudy	15	98	18	217
Sept. 28, 18°, sun	56	92	30	330
Oct. 15, 19°, cloudy	71	90	7	500
Nov. 23, 7°, cloudy	0	100	.7	1,660

[1] All were made with Perfection kerosene at the rate of 250 gallons an acre.

The applications on September 28 in sunny weather were less effective than those made 3 days earlier on a cloudy day, but were no less effective than applications made on a cloudy day in mid-October. The results suggest that sunshine does not affect the value of the spray, at least at the lower temperatures, except as it increases the vaporization of the kerosene before it has penetrated into the roots of the dandelion plants.

In contrast to the serious grass injury observed in plots sprayed November 23 at 7° C. (table 6), no permanent injury was noted in a

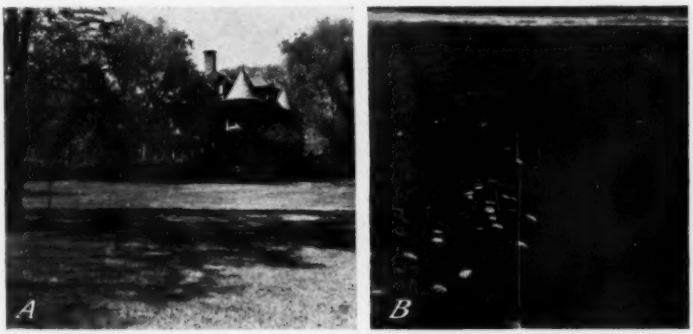

FIGURE 3.—The control of dandelions with kerosene: *A*, A 99-percent control without grass injury in a lawn sprayed October 17, 1936; *B*, persistence of control in plots at right given one spray 2 years before photograph was taken.

series of 32 plots sprayed at the same temperature on September 30, 1934, and the degree of dandelion control was the same (99 to 100 percent) in both tests. All the observations on temperature may be summarized by saying that good results were obtained at temperatures up to 23° by spraying in the evening or on cloudy days. The usual result of higher temperature has been reduced effectiveness, and occasionally greater grass injury as well. Temperatures down to freezing have not prevented good results when other factors were favorable. In a large area sprayed October 17, 1936, with a power sprayer, no grass injury was observed the following June on exposed locations (fig. 3, *A*), but injury amounting to complete kill was noted on steep north slopes of the same area and in the shade of evergreen trees. Dandelion control was better than 98 percent throughout the

plot. These results, in connection with those given above, suggest that a certain amount of warm weather after spraying reduces the kerosene residue in the sod by evaporation, and prevents permanent injury. A higher temperature, particularly at the time of spraying and in combination with bright sunshine, causes excessive evaporation and reduces the quantity of kerosene reaching the dandelion roots to less than the lethal dose.

Seasonal Factors

The larger differences in temperature have been obtained, in most cases, by varying the season of application. Kerosene applications have been made at frequent intervals between May 1 and December 1 with good control in at least some of the plots. Early spring treatments (as soon as the dandelions are well up) have given good control of old plants and satisfactory final results on plots with a good stand of grass. When the dandelions were thick and the grass thin, too much bare area was left on which dandelion seedlings became established during late spring. As tables 5 and 6 show, treatments that injured the grass were followed by high seedling counts.

Midsummer applications of kerosene are difficult to make on a large scale because the work must be done in cloudy weather. Late fall applications have resulted in serious winter injury to the grass (table 6). Early fall applications (September 15 to October 10 at Ames) have given the best results. Two factors appear to be involved—the more generally moderate temperatures, and the double growing season, both fall and spring, for the closing of the bared areas by stolon growth of the grass. Sprayed bluegrass makes no growth for 2 to 3 weeks after the treatment. Fall applications should allow for 4 to 6 weeks of normal growth after this temporary set-back; that is, sprays should be made 6 weeks or 2 months before the expected date of grass-killing freezes or deep snow.

THE PHYSIOLOGICAL ACTION OF KEROSENE

Dandelions sprayed with kerosene develop a dark-green color within a few seconds as the oil penetrates the intercellular spaces of the leaves. The dark color disappears within 24 to 36 hours, and for the next 6 to 20 days the plants appear normal to yellowish. The odor of kerosene usually can be detected on root fragments during this period; the phloem becomes translucent and the quantity of latex exuded from a cut root surface increases. The first conspicuous symptom of injury is a reddening or bronzing of the leaves which appears about 1 week after spraying in warm weather and 2 to 3 weeks after spraying in cooler weather. This change in color was accompanied, as shown by the data in table 7, by a fourteenfold increase of sucrose in the leaves. Polysaccharides (levulins plus dextrin), reducing sugars, and soluble nitrogen showed moderate increases in the leaves of the sprayed plants in the 2 weeks after treatment. Changes in the root consisted principally of a digestion of polysaccharide to reducing sugars with less change in sucrose and total carbohydrates. The slow digestion of the reserves of the root and the rapid accumulation of carbohydrates, particularly sucrose, in the leaves may be considered as indications of interference with translocation. This conclusion was supported by histological studies which showed necrosis of the phloem in the roots of the sprayed plants and the accumulation of

TABLE 7.—*The chemical composition on May 20 and June 1 of the leaves and roots of nonsprayed dandelion and of dandelion sprayed with kerosene on May 18, 1935*

Date of sampling and fraction	Leaves		Roots	
	Control	Sprayed	Control	Sprayed
May 20, 1935:	*Percent*	*Percent*	*Percent*	*Percent*
Reducing sugars	0.40	0.28	0.13	0.17
Sucrose	.23	.45	.69	.35
Polysaccharides	1.01	1.05	2.66	2.87
Soluble nitrogen	.03	.03	.04	.04
Total nitrogen	.31	.27	.27	.21
June 1, 1935:				
Reducing sugars	.24	.64	.33	1.03
Sucrose	.16	2.43	1.09	1.04
Polysaccharides	1.16	1.58	3.38	2.71
Soluble nitrogen	.02	.03	.04	.05
Total nitrogen	.18	.16	.15	.17

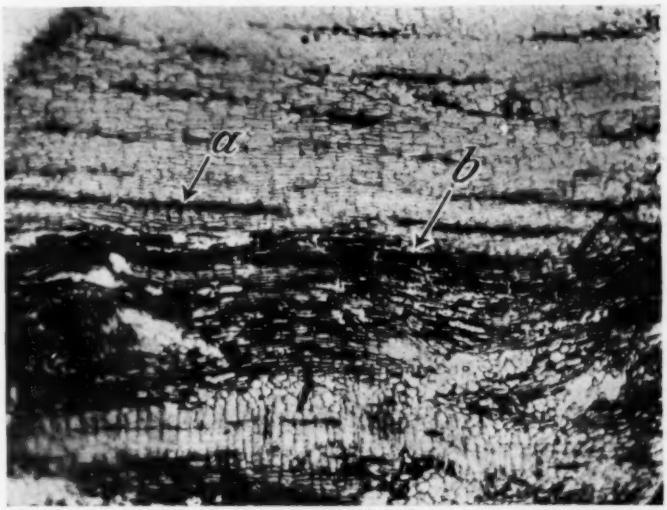

FIGURE 4.—Kerosene injury in dandelion root: Phloem necrosis at *a*, and plugging of outer xylem with latex at *b*.

latex (fig. 4) in the outer vessels. Injury was commonly observable to depths of 30 cm, and the death of the tops appeared to be due to the previous death of the roots.

Bluegrass (both *Poa pratensis* and *P. compressa*), white clover (*Trifolium repens*), common plantain (*Plantago major*), and buckhorn (*P. lanceolata*) have not been seriously injured by kerosene sprays applied under the conditions recommended. Foxtail (*Setaria* spp.) and crabgrass (*Digitaria* spp.) have shown more injury than bluegrass, but have survived in some treatments. The action of kerosene is thus seen to be rather specific for dandelion. Possibly the leafy rosette and fleshy taproot of this plant are favorable to the absorption and retention of the oil. Even under favorable conditions, bluegrass may be killed by appreciably heavier applications than the 200 gallons an

acre recommended, or under conditions unusually favorable to the persistence of the oil within the bluegrass plants, and a greater accumulation and longer retention of the kerosene in the dandelion root would appear to be the most probable explanation of the greater toxicity of the light applications on this plant. A second factor in differential killing is the ability of isolated bluegrass buds to produce roots and grow, even when much of the rhizome system has been destroyed, whereas dandelion plants do not recover after the taproot is killed.

THE CARE OF TREATED LAWNS

The heavy seeding and ready distribution of the dandelion fruit insure the presence of the plant in any available area. However, the seedlings do not compete with well-established sod, particularly in moderately dry weather. Figure 3, *B*, shows a Canada bluegrass (*Poa compressa*) sod which was still practically free of dandelions 2 years after it had received a single kerosene spray. This plot was fertilized twice during this interval and observations showed that the many dandelion seedlings which had started on the area each spring died during July and August before their root systems had penetrated below the level of the grass roots.

The sensitivity of dandelion seedlings to dry weather is shown by the behavior of the seedlings on the plots sprayed November 23, 1935, which were estimated to have an average of 1,660 seedlings per 100 square feet the following May (table 6). Less than 10 dandelions per plot, or about 0.5 percent, of these seedlings survived the drought of 1936. Older, deeper rooted plants are seldom injured by dry weather and more than 80 percent of the large plants on the check plots survived the 1936 drought.

Injudicious sprinkling during the summer months will aid in keeping dandelion seedlings alive and will hasten reinfestation. Ammonium sulphate fertilization (*12*) and ferrous sulphate sprays, heavy but infrequent watering, and less mowing during dry weather with the mower set high at all times, will retard reinfestation. Some spotting work with a kerosene spray will be required at intervals to remove plants which become established in breaks in the sod.

Especial care is required to insure the rapid resodding of lawns in which large numbers of dandelions have been killed and much of the ground left bare. Reseeding is rarely successful because dandelion and grass seedlings develop under similar conditions. Natural spread of the grass by stolons must, therefore, be depended upon. Frequently a year of treatment with ammonia fertilization and iron sulphate spraying should precede the kerosene to encourage the establishment of grass between the dandelion plants. With a month of cool moist weather, good sods may be reestablished, if there is grass among the dandelions, by applying ammonium sulphate at the rate of 150 to 300 pounds per acre and phosphorus and potassium as needed. Good sod has been established without reseeding in a single season from scattered bluegrass plants which covered less than 1 percent of the area in the spring.

One factor in the handling of sprayed lawns that needs further investigation is the matter of early spring mowing. The recent emphasis on the importance of reserves in grasses (*9*, *11*) has led to the belief that spring cutting or pasturing of bluegrass should be delayed.

Graber (*10*) has shown, however, that close cutting of fertilized sod allowed to grow tall may seriously check the development of the grass, and Leukel and Coleman (*14*) have shown that Bahia grass allowed to grow naturally and to produce seed made a much poorer sod than grass cut at intervals. A successful lawn cultural practice should maintain a neat appearance, and moderately close mowing at some stage is necessary. It seems probable that spring mowings should be made early enough and frequently enough to prevent seed formation or the shading out of the lower leaves of the grass. If the mower is set to cut at a height of 1½ to 2 inches, frequent cutting will encourage the development of leaves near the ground and will permit full leaf coverage for photosynthesis while keeping the lawn neat and smooth.

In contrast to this spring treatment, the lawn should be mowed infrequently or not at all during dry weather, and mowing should stop a month before the end of the growing season in the fall to encourage the accumulation of reserves for winter.

SUMMARY

The food reserves of the common dandelion (*Taraxacum officinale*) are shown to consist largely of a mixture of levulin and dextrin, and to have reached a low level for the samples tested during early spring growth.

Straight-run kerosene with a boiling-point range of 180° to 250° C. and an unsaturated hydrocarbon content of not over 4 percent has been shown to be a very effective differential spray for dandelions in bluegrass-white clover lawns. The undiluted kerosene is sprayed uniformly over the lawn during cool, cloudy weather, at the rate of 200 gallons an acre. The best results have been obtained by spraying 2 months before the end of the bluegrass growing season, or September 15 to 30 at Ames, Iowa. The kerosene appears to accumulate and to be retained in the taproot of the dandelion and to affect the plant through the destruction of the phloem of the root. Other common lawn plants and weeds show little or no injury from applications of kerosene which are effective against dandelion. Scattered dandelion plants may be eradicated at any time by spraying the individual plants lightly with kerosene.

Bluegrass as well as dandelions will be killed (1) if more than about 300 gallons of kerosene are used instead of the recommended 200; (2) if furnace or tractor distillates are used instead of kerosene; and (3) if the spray is applied too late in the fall to permit the grass to make a partial recovery before winter.

The rapid reestablishment and maintenance of a close sod with the avoidance of frequent, light waterings during dry periods are important in keeping dandelions from becoming established in lawns. The dandelion seedlings will not survive a dry period of moderate intensity when growing in heavy sod, but they may become established in open areas or if sprinkled frequently. Older plants with roots below the level of the grass roots should be eradicated with kerosene.

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INFLUENCE OF CERTAIN ENVIRONMENTAL CONDITIONS ON CONGESTION OF STARCH IN TOMATO PLANT STEMS [1]

By ARTHUR C. FOSTER, *senior pathologist*, and EVERETT C. TATMAN, *assistant scientific aide, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The phenomena of photosynthesis, the synthesis of the simpler carbohydrates, the translocation of these materials to all parts of the plant, and their further transformation into starch to be stored have been subjects of speculation and experimentation for many years. Since starch is one of the most widely distributed plant products, investigators have been considerably interested in determining the relation of various factors, such as mineral elements and environmental conditions, to the accumulation or congestion of starch in various parts of the plant. The relation of potash to the formation of starch in plants and its subsequent hydrolysis when needed for further growth has received considerable attention, but specific tangible results appear to have been difficult to obtain. The part that potash plays in the formation and utilization of starch is uncertain, but it presumably acts as a catalyst and has radioactive properties (*4*).[2] An enormous amount of data has been presented in the literature which demonstrates clearly that nitrogen deficiency plays a very definite part in the accumulation of starch or other carbohydrates in the plant. High nitrogen content is generally accompanied by medium to low carbohydrate content, and very low nitrogen usually indicates high carbohydrate accumulation.

A number of investigators have demonstrated rather definitely that starch accumulation, or congestion, takes place in tomato plants under conditions of deficient nutrient supply or under environmental conditions that are accompanied by retardation of growth. However, there appear to be very few quantitative data in the literature showing the interaction of fertilizer deficiency or nitrogen deficiency and other unfavorable environmental conditions, such as soil moisture and temperature, in relation to the congestion of starch in the tomato plant.

The present paper presents data showing the influence of fertilizers, soil moisture, temperature and humidity, and evaporating power of the air on the accumulation of starch in the stems of tomato plants (*Lycopersicon esculentum* Mill.). The data were secured incidentally in a study of the effect of environmental conditions on the growth responses of tomatoes as related to the appearance of certain nonparasitic diseases and other abnormal physiological disturbances.

REVIEW OF LITERATURE

There is an extensive literature that relates to the various factors influencing the accumulation of the simpler carbohydrates and the

[1] Received for publication October 1, 1937; issued June 1938.
[2] Reference is made by number (italic) to Literature Cited, p. 881.

total hydrolyzable polysaccharides, but that relating to congestion of starch as such is not extensive. Hartwell (*2*) appears to have first recognized the effects of unfavorable conditions on the congestion of starch in potatoes and beets. At first he was of the opinion that starch congestion was due mainly to potash deficiency, but later he came to the conclusion that any condition unfavorable to plant growth would produce the phenomenon. Unfortunately, Hartwell did not secure quantitative data, but instead he made a microscopic estimation of the starch present. Kraus and Kraybill (*5*), in their work on vegetation and reproduction, presented extensive data to show that starch accumulates in tomato stems when the plants are grown with a deficient nitrogen supply. Jansen and Bartholomew (*3*) found that the amount of potassium present in the plant was correlated with the total sugars, but they seemed to be doubtful as to its relation to starch accumulation or congestion. In addition to the above citations, a large amount of work has been recorded in the literature showing the effects of low temperature on the accumulation of polysaccharides, on the synthesis of nitrogen compounds, and on general metabolism in the plant. The work of Nightingale (*6*) on the effects of temperature on metabolism and accumulation of carbohydrates in tomatoes demonstrated the responses of plants to different temperatures, but he, as well as others, apparently did not consider it necessary to maintain accurately controlled soil moisture. There seems to have been a rather general lack of appreciation of the very marked differences in growth and physiological behavior that result from relatively small differences in water supply.

METHODS AND MATERIALS

Experiments were conducted at the Arlington Experiment Farm, Arlington, Va. The methods of procedure were rather unique. The tomato plants were grown in a good quality of greenhouse soil made up of loam from the Arlington farm, composted with manure and muck, to which sand was later added. All soil before being used was passed through a ¼-inch-mesh wire screen to insure uniformity. This soil had a water-retaining capacity of 65 percent of its dry weight, and the water-retaining capacity remained uniform over a period of 5 years of experimental work.

The plants were grown at 3 soil-moisture levels of approximately 38, 47, and 56 percent of the dry weight of the soil. On a water-retaining-capacity basis, these were, respectively, 58, 72, and 86 percent. These series were set up by first determining the original amount of water in the soil and then adding sufficient water to bring the water content up to the required percentage. The soil moisture was maintained at approximately the desired level by frequent additions (four to six times daily) of measured amounts of water, the required amounts being determined by weighing the cultures on solution balances of 40-kg capacity.

All plants were grown in 3-gallon glazed crocks holding 12 kg of soil. As the soil was placed in the crocks, fertilizer containing various proportions of nitrate of soda, superphosphate, and potassium sulphate was mixed with it at the rate of 2 tons per acre. Three fertilizer formulas were used, namely, 12–0–12, 12–6–12, and 6–12–6, in the

order of nitrogen, phosphorus, and potassium, respectively; the checks received no fertilizer treatment.

Five crocks of each fertilizer treatment were placed in each of the three soil-moisture series, and these were replicated in each of three different temperature units of the greenhouse. This gave for study 5 replications at each of the 36 points of observation and a total of 180 plants under almost perfectly controlled environmental conditions.

The seeds (Marglobe variety), which were first sown in flats August 15, 1934, were transplanted to 2½-inch pots on August 25 and to the 3-gallon crocks on September 4, where they were allowed to become established. The plants were staked with heavy wire and later, with the appearance of the first flower cluster, the wire stakes were replaced

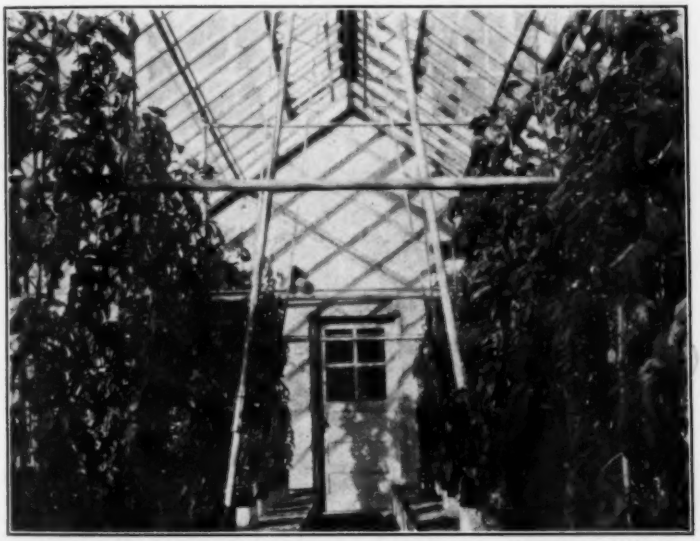

FIGURE 1.—High-temperature greenhouse, unit 1, with daily mean temperature of 74° F. Note tall spindling growth habit of tomato plants, due mainly to high soil moisture and high temperatures.

with 6-foot bamboo stakes. The plants were pruned to a single stem, tied to the stakes, and eventually topped after the appearance of the sixth flower cluster. During the growth period, the moisture content of the soil was kept approximately constant by the method already mentioned. A careful record was kept of the amount of water consumed by each plant daily for the duration of the experiment.

GROWTH HABIT AND RESPONSE OF TOMATO PLANTS TO ENVIRONMENT

The influence of temperature on the growth response and habit of tomato plants is shown in figures 1, 2, 3, and 4, which are from photographs of the mature plants grown in the three different greenhouse temperature units. In the high-temperature unit (fig. 1) the plants

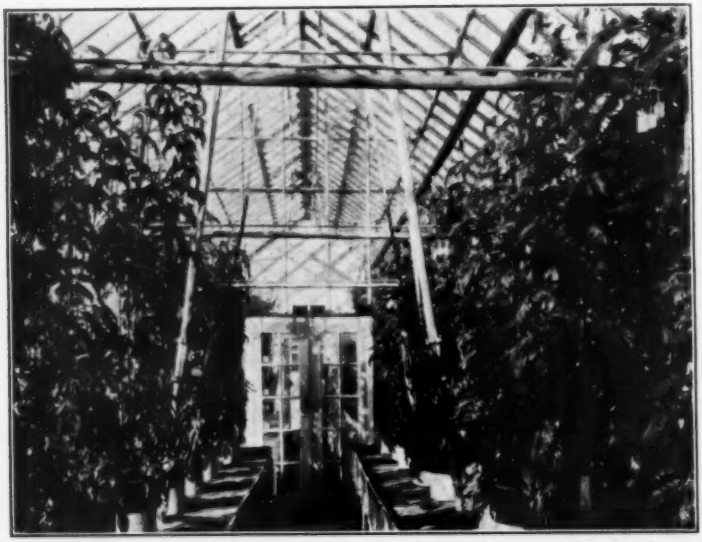

FIGURE 2.—Medium-temperature greenhouse, unit 2, with daily mean temperature of 70° F. Tomato plants not so tall as at 74°, but stockier, with thicker leaf growth.

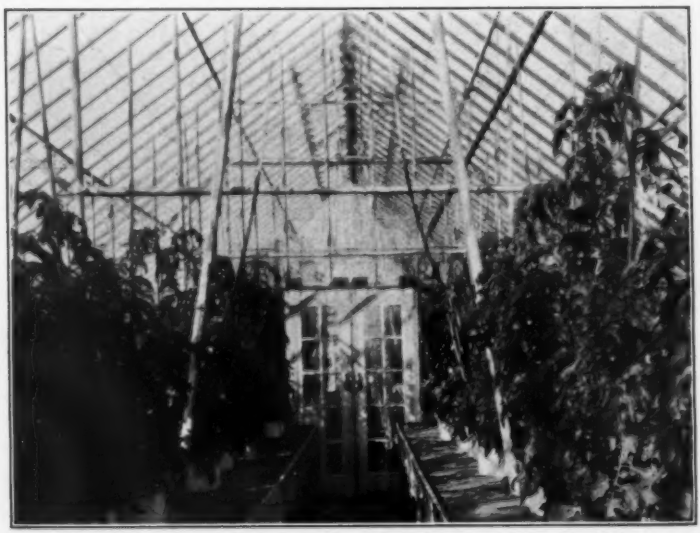

FIGURE 3.—Low-temperature greenhouse, unit 3, with daily mean temperature of 65° F. Note short, stocky growth habit of tomato plants, with heavy stems and much heavier leaves than those shown in figures 1 and 2.

were tall and slender with relatively small stems and long internodes; they were practically barren, succulent, and of the spindling

FIGURE 4.—Tomato plants showing influence of different temperatures on growth. Cultures grown at (*A*) 65°, (*B*) 70°, and (*C*) 74° F. All grown at 86 percent of soil-water-retaining capacity and with 12-6-12 fertilizer.

type (fig. 4, *C*). In the medium-temperature unit (fig. 2) the plants were shorter, stems were heavier, and leaves were spaced closer together. The plants had better color and vigor than those in the

higher temperature unit, and they were quite fruitful (fig. 4, *B*). In the lowest temperature unit (fig. 3) the plants were considerably shorter, had dense green foliage (fig. 4, *A*), and the appearance of being very succulent, although they contained much larger percentages of dry material than those of the other units. In figure 5 is illustrated the influence of soil moisture on plant growth and habit when other conditions are uniform. These plants were grown in the 1932 series of experiments, at 76° F. mean temperature, and

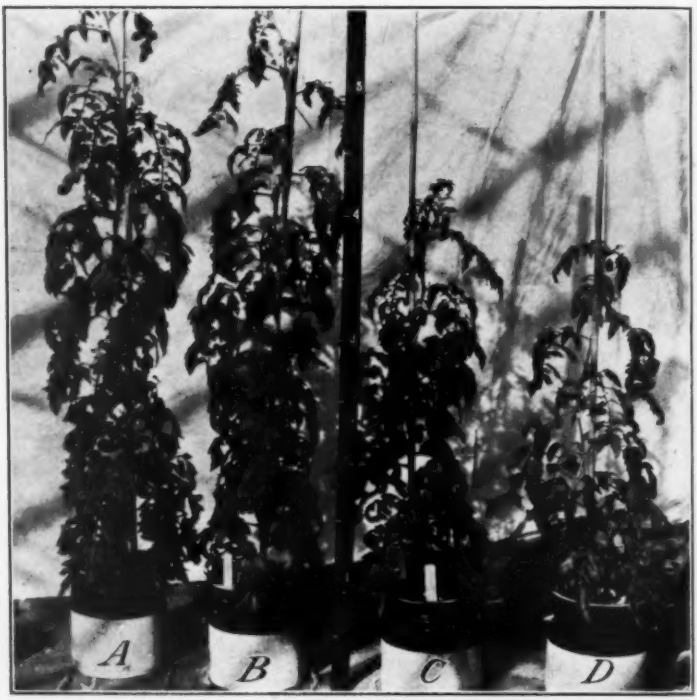

FIGURE 5.—Tomato plants showing influence of soil moisture on growth. Cultures grown at (*A*) 65, (*B*) 56, (*C*) 47, and (*D*) 39 percent of soil-water-retaining capacity. All grown at 76° F. and with 12-2-2 fertilizer. Plants from a series grown in 1932.

treated with a 12–2–2 fertilizer. Figure 4 shows the very marked influence of temperature on growth and habit of the tomato plants, all other conditions being uniform, with soil moisture at 86 percent of water-retaining capacity, and 12–6–12 fertilizer.

It was intended to operate the greenhouse units at 75°, 70°, and 65° F., respectively, from the beginning of the experiment, September 10, but high outside temperatures made it impossible to keep the units at these temperatures until about the first week in October. Table 1 shows the weekly mean temperatures for the three units as calculated by planimeter readings from hygrothermograph charts.

For the duration of the experiment the mean temperatures were 74.3°, 70.4°, and 65.7° in units 1, 2, and 3, respectively. From October 2 to December 17, during which period quite constant temperature differences could be maintained, the means were 74.6°, 69.1°, and 62.9°, respectively.

The mean relative humidities of units 1, 2, and 3 for the entire period were 55.3, 60.0, and 63.1 percent, respectively. This order of difference existed throughout the period regardless of the temperature, probably as a result of differences in ventilation incidental to attempts to regulate temperatures.

The data of the atmometric indexes of the three greenhouse units are summarized and presented in table 2.

TABLE 1.—*Mean temperatures and average relative humidities of three greenhouse units in which the cultures were grown, 1934*

Week ended	Weekly mean temperature			Relative humidity		
	Unit 1	Unit 2	Unit 3	Unit 1	Unit 2	Unit 3
	° *F.*	° *F.*	° *F.*	*Percent*	*Percent*	*Percent*
Sept. 10	73.5	74.5	74.0	61.5	67.3	69.9
Sept. 17	75.1	76.0	75.2	64.4	68.7	72.6
Sept. 24	71.1	71.2	71.4	49.6	62.8	66.2
Oct. 1	73.5	74.0	73.4	59.7	63.6	65.2
Oct. 8	68.9	66.9	65.8	56.3	60.0	62.6
Oct. 15	73.5	69.6	66.8	49.8	54.5	55.5
Oct. 22	75.1	71.5	65.0	53.9	58.0	60.6
Oct. 29	75.0	69.4	63.0	54.9	57.5	62.5
Nov. 5	74.3	69.7	62.2	54.1	58.0	60.3
Nov. 12	75.3	68.8	61.7	52.6	58.5	60. .
Nov. 19	76.3	68.8	61.6	52.4	54.5	55.8
Nov. 26	75.8	70.2	63.4	59.5	62.7	68.0
Dec. 3	76.0	69.6	62.9	64.0	69.8	75.3
Dec. 10	74.0	67.4	59.7	50.8	55.0	59.5
Dec. 17	77.1	68.1	60.1	45.9	48.7	52.4
Average	74.3	70.4	65.7	55.3	60.0	63.1

TABLE 2.—*Effects of certain environments on congestion of starch in tomato plant stems, and the relation of plant and fruit growth, water requirement, and chemical composition on a dry-weight basis for the 1934 crop*

Soil moisture (percent)	Fertilizer formula	Unit 1.—Average daily mean temperature, 74° F.; relative humidity, 55 percent; corrected evaporation from atmometers, black 19.8 ml, white 14.8 ml, difference 5 ml						
		Dry weight of plants	Weight of green fruit	Water requirement [1]	Dry material [2]	Polysaccharides	Starch	Total nitrogen
		Grams	*Grams*	*Cc*	*Percent*	*Percent*	*Percent*	*Percent*
38 [3]	12-0-12	32.9	585	579	12.09	16.06	1.54	1.22
	12-6-12	30.2	670	559	12.74	15.70	1.68	1.27
	6-12-6	44.1	910	572	11.98	16.70	1.59	1.25
	Check	46.0	790	668	11.36	14.53	1.27	.99
47 [4]	12-0-12	63.0	1,180	629	11.11	14.38	.62	1.25
	12-6-12	64.3	735	620	9.22	14.53	1.21	1.74
	6-12-6	92.5	1,745	678	12.47	14.68	1.58	.50
	Check	84.0	1,400	721	14.63	21.88	9.40	.44
56 [5]	12-0-12	118.3	1,585	694	10.37	14.38	.46	1.18
	12-6-12	92.9	2,145	685	10.49	15.08	1.33	.97
	6-12-6	99.2	2,120	721	8.56	18.80	1.90	.49
	Check	94.9	1,385	786	12.66	17.62	3.87	.23

See footnotes at end of table.

TABLE 2.—*Effects of certain environments on congestion of starch in tomato plant stems, and the relation of plant and fruit growth, water requirement, and chemical composition on a dry-weight basis for the 1934 crop*—Continued

Soil moisture (percent)	Fertilizer formula	Unit 2.—Average daily mean temperature, 70° F.; relative humidity, 60 percent; corrected evaporation from atmometers, black 16.5 ml, white 14.0 ml, difference 2.5 ml						
		Dry weight of plants	Weight of green fruit	Water requirement [1]	Dry material [2]	Polysaccharides	Starch	Total nitrogen
		Grams	*Grams*	*Cc*	*Percent*	*Percent*	*Percent*	*Percent*
38 [3]	12–0–12	34.6	700	450	13.58	15.99	2.90	1.24
	12–6–12	24.9	480	398	12.24	16.18	1.94	1.38
	6–12–6	32.6	840	491	12.33	15.98	2.42	1.27
	Check	69.1	910	632	16.69	23.68	11.56	.63
47 [4]	12–0–12	68.2	1,215	493	10.13	14.98	1.67	1.39
	12–6–12	72.0	1,320	472	10.35	14.50	1.92	1.38
	6–12–6	81.6	1,555	535	11.66	14.60	1.65	.91
	Check	97.3	1,175	648	15.51	23.98	12.70	.22
56 [5]	12–0–12	98.9	1,555	558	8.64	14.70	.58	1.41
	12–6–12	87.1	1,515	547	9.01	14.76	2.19	1.28
	6–12–6	92.8	1,840	618	10.86	14.66	1.82	.70
	Check	96.7	1,255	721	13.00	18.91	6.14	.59

Soil moisture (percent)	Fertilizer formula	Unit 3.—Average daily mean temperature, 65° F.; relative humidity, 63 percent; corrected evaporation from atmometers, black 15.1 ml, white 12.7 ml, difference 2.4 ml						
		Dry weight of plants	Weight of green fruit	Water requirement [1]	Dry material [2]	Polysaccharides	Starch	Total nitrogen
		Grams	*Grams*	*Cc*	*Percent*	*Percent*	*Percent*	*Percent*
38 [3]	12–0–12	35.6	225	317	12.41	20.62	8.49	1.35
	12–6–12	54.3	150	302	12.26	19.18	5.61	1.42
	6–12–6	68.1	650	446	12.45	19.50	5.55	.91
	Check	84.8	910	535	20.36	33.65	22.90	.21
47 [4]	12–0–12	78.2	850	409	10.66	14.35	3.11	1.34
	12–6–12	84.5	845	336	9.78	14.92	2.39	1.54
	6–12–6	113.5	655	452	10.02	14.98	3.64	1.23
	Check	97.4	1,085	551	17.95	35.17	24.10	.36
56 [5]	12–0–12	116.3	1,110	445	8.69	11.86	1.09	1.70
	12–6–12	73.1	325	438	7.53	13.28	2.32	2.22
	6–12–6	121.0	1,455	486	10.97	18.51	6.32	.46
	Check	106.8	1,345	590	17.35	31.10	19.52	.31

[1] Per gram dry weight of tops.
[2] Percentage of fresh weight.
[3] 58 percent of water-retaining capacity.
[4] 72 percent of water-retaining capacity.
[5] 86 percent of water-retaining capacity.

It is important to note that in growth and general vigor the plants used in all of these experiments were similar in size and character to what might be expected under field conditions. The plant growth approached normal, and it is for this reason that the results to be presented are unique and distinct from those of similar lines of work reported by other investigators, who have used sand or solution cultures that resulted in stunted and abnormal growth.

CHEMICAL METHODS

The samples for chemical analyses of the stems were collected on December 12, 1934, from mature plants of the types illustrated in figures 1, 2, and 3. The leaves were previously removed and preserved in 75-percent alcohol, and 100-g samples of the top parts of the stems were sliced into 75-percent alcohol. To insure uniformity of samples, all collections were made early in the morning on cloudy or rainy days. In the collection, preparation, and preservation of samples in alcohol, essentially the same methods were followed as were used by Boswell (*1*). In the further preparation of the samples for analysis, Boswell's methods were also followed, except that the dry material was ground in a Wiley type laboratory mill, F. R. I. model, to pass through a 100-mesh wire screen. In making the starch deter-

minations, the method developed by Walton and Coe (7) was used. It proved to be very satisfactory for this type of material, and duplicate or triplicate determinations consistently were in good agreement.

INFLUENCE OF ENVIRONMENT ON WATER REQUIREMENT OF THE TOMATO

To present a better conception of the growth responses of the tomato plants grown under different environmental conditions, a large amount of data was collected on the relative transpiration rates of the different cultures. The term "water requirement" of plants has been used by many investigators to express the ratio between water absorbed and dry matter produced. It is usually obtained by dividing the total units of water absorbed by the plant by the total units of dry matter produced. The data presented in table 2 on water requirement do not represent "water requirement" as generally defined, but indicate rather the relative transpiration rate of the plants in the several environments. This transpiration rate is determined by dividing the total units of water absorbed or transpired by the dry weight of the aerial vegetative part of the plants rather than by the total dry weight of all plant parts. It is well known that many factors may influence the water requirement of plants, which varies widely with different species.

As is shown by the data presented in table 2, variation in environmental conditions such as fertilizers, soil moisture, humidity, and air temperature may cause a wide variation in the efficient use of water by the plant. Low soil moisture and low air temperature have great influence in reducing the water consumption in proportion to the dry material produced; furthermore, increasing amounts of nitrogen promote greater efficiency in water utilization. In these studies plants without any added fertilizer were the most extravagant users of water.

EFFECTS OF FERTILIZERS ON ACCUMULATION OF STARCH IN TOMATO STEMS

The excessive accumulation of starch in the stems of the check plants may appear unusual and perhaps difficult to accept as truly representative, but study of the data in table 2 shows a consistent trend in the results and supports these observations. The check plants received no fertilizer, but the soil used was fairly fertile because a small amount of well-rotted manure had been added. However, a decided change in color and retarded growth indicated that these plants had used nearly all of the readily-available nitrogen when they were about half-grown. Under certain environmental conditions the accumulation of starch was most marked in the check plants; however, these plants, when grown with low soil moisture at high temperature, had less starch than the fertilized plants. We might accept the explanation that under these conditions the storage carbohydrates were largely consumed in respiration and that poor nutrition retarded their replacement. At the lowest temperature, there was marked congestion of starch in the stems even with the low soil moisture.

It is evident from these and other studies that under these experimental conditions starch congestion is more definitely related to low nitrogen supply than to differences in potash supply. No evidence of potash deficiency has been observed in any of the cultures grown

with this greenhouse soil over a period of 5 years, but numerous instances have been observed of definite exhaustion of available nitrogen. It is also apparent that other environmental factors exert considerable influence, and these will be discussed later. The influence of very marked plant-food deficiency on starch congestion has been discussed in detail by many investigators; however, little consideration has been given to the effects of approximate soil-moisture differences on plants of normal size and appearance grown in good, fertile soil.

EFFECT OF TEMPERATURE ON ACCUMULATION OF STARCH IN STEMS

The effect of temperature on the congestion of starch in stems of nitrogen-starved plants is very marked, as is shown in figure 6.

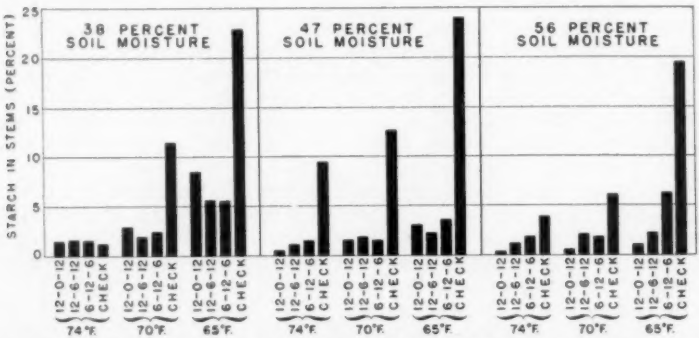

FIGURE 6.—Influence of temperature on accumulation of starch in tomato stems in various fertilized and in unfertilized cultures grown at three different levels of soil moisture.

In the 38-percent soil-moisture, no-fertilizer cultures grown in the high-temperature unit (74° F.) there was 1.27 percent of starch, an amount even lower than in any of the fertilized treatments in the same moisture and temperature series; but at 70° the starch content was 11.56 percent. At a still lower temperature (65°), the starch content was 22.9 percent, or approximately 18 times as much as at the high temperature, owing chiefly to a reduction of 9° in daily mean temperature. A difference in temperature of 9° also shows a rather significant but less marked influence on the congestion of starch in the fertilized plants in this low soil-moisture series, as further examination of the data in table 2 and figure 6 reveals.

It is interesting that 47 percent of soil moisture appeared to be optimum for starch accumulation at each temperature level in the unfertilized or check plants. However, the fertilized plants in this soil-moisture series showed relatively little fluctuation in starch content due to temperature difference. As in the case of the 47-percent soil-moisture series, the significant influence of relatively low temperature was markedly apparent in the 38- and 56-percent series (fig. 6). The temperature influence was much greater in the nitrogen-starved or check plants than in the fertilized series. In the 47-percent soil-moisture check cultures the percentage of starch was 9.4 at the high temperature, 12.7 at the medium temperature, and much higher still,

24.1, at the low temperature, or nearly three times the amount found in plants grown at the high temperature.

EFFECT OF SOIL MOISTURE ON ACCUMULATION OF STARCH IN STEMS

In figure 7 is shown the influence of soil-moisture supply on the congestion of starch in tomato stems. Differences in soil-moisture supply appear to have a very definite and significant influence on the congestion of starch in the stems, with 47 percent shown to be optimum for the check plants at each experimental temperature in these tests. No plausible explanation can be offered for this unless we consider that 47 percent of soil moisture (72 percent of the water-retaining capacity) was optimum for plant growth, and because of this condition the check plants exhausted their available nitrogen supply and accumulated more starch than the others. The wide variation in the amount of starch with respect to soil moisture in the fertilized series at the low temperature is probably due to the effect of this temperature on the

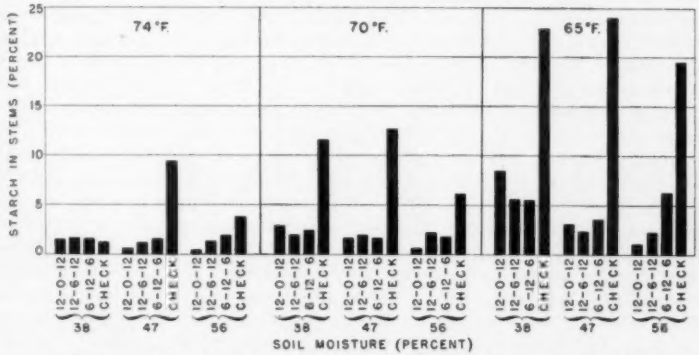

FIGURE 7.—Influence of soil moisture on accumulation of starch in tomato stems in various fertilized and in unfertilized cultures grown at three uniform temperatures.

growth activities of the plants, especially protein formation, cell division, and metabolic activity (*6*). However, since the plant response as characterized by starch congestion is so varied, a better knowledge of the interrelation and interaction of the numerous factors is necessary before an explanation can be attempted. Additional data obtained in these studies and not yet analyzed should aid in a further understanding of them.

DISCUSSION

The data presented in this paper confirm further the results obtained by Hartwell (*2*), Kraus and Kraybill (*5*), Nightingale (*6*), and many other investigators along similar lines, many of whom, however, have used different crops or methods. The experimental methods followed are similar to those followed by Nightingale (*6*) in growing tomato plants, with two major exceptions: (1) He used sand cultures and nutrient solutions, and (2) he made no attempt to determine the relation of soil-moisture supply to starch congestion or accumulation. The extreme variation in temperature conditions in the present experimental work was only 9° F., while in Nightingale's it was 41° (from 55° to 96°). The general growth habit, vigor, and plant response of

the writers' plants approached what might be called normal for field conditions (figs. 1, 2, 3, 4, and 5), while Nightingale's were stunted in comparison.

The response of the plants to the four fertilizer, three soil-moisture, and three temperature conditions, and the accumulation of starch resulting from the interaction of the various combinations of these factors, are highly significant, as shown by analysis of variance. Differences in starch congestion or accumulation at any one temperature were apparently influenced almost entirely by total nutrient deficiency and were apparently unrelated to marked differences in potash supply. In the low-temperature unit, when nitrogen metabolism and synthesis of proteins were retarded because of unfavorable temperature, there was a high accumulation of starch in the plants even though they received large amounts of nitrates. A different explanation is suggested for the results on high-nitrogen versus unfertilized check plants in the low-moisture series, when grown in the high-temperature unit. In this case the low soil moisture apparently retarded plant growth, which tended to conserve the available nutrient supply for use by the plant over a longer period; and since nutrient deficiency did not occur, accumulation of starch was about the same as in the fertilized plants. The condition of nutrient deficiency, due either to the exhaustion of the available supply or to failure on the part of the plant to assimilate because of low temperatures, shows a highly significant relation to the congestion or accumulation of starch.

The relation of low temperatures to starch congestion was clearly demonstrated by Nightingale (*6*). However, under his experimental conditions he could not observe the highly significant relation of soil-moisture supply to starch congestion. The fact that in the present experiments 47 percent of soil moisture gave the greatest starch accumulation or congestion for the unfertilized plants in each of the three different temperature units is illustrated in figure 7. This condition is thought to be due to the moisture percentage (72 percent of the water-retaining capacity) being the best of the three for plant growth; this resulted in early exhaustion of the available nitrogen, with consequent larger accumulation of starch than occurred under other moisture conditions. Further discussion of the interrelation and interaction of the various factors concerned in metabolism and synthesis and of the relation of these processes to starch congestion would best be deferred until there is a better understanding of these problems. This must await further analysis of data and perhaps further experimentation.

SUMMARY

An intensive study has been made of starch congestion or accumulation in stems of tomato plants, their growth response to various environmental influences, and the interrelation and interaction of these factors as influenced by (1) four soil-fertilizer treatments, (2) three soil-moisture conditions, and (3) three air-temperature conditions.

Starch congestion appears to be more definitely associated with nitrogen deficiency than with wide variation in potash supply, since numerous cultures high in starch have shown typical symptoms of nitrogen deficiency but no symptoms of potash deficiency.

In the unfertilized plants of the 38-percent soil-moisture series, with a decided lowering in temperature (from 74° to 65° F.) the congestion of starch increased from 1.27 to 22.9 percent.

In the unfertilized plants of the 47-percent soil-moisture series, a similar drop in temperature caused an increase from 9.4 to 24.1 percent in starch.

In the unfertilized plants of the 56-percent soil-moisture series, a corresponding drop in temperature caused an increase from 3.87 to 19.52 percent of starch.

The same lowering of temperature (9° F.) also caused an increase of starch in the fertilized plants. When the plants were grown with 38 percent soil moisture starch increased from 1.54 to 8.49 percent for the 12–0–12 fertilizer, from 1.68 to 5.61 percent for the 12–6–12 fertilizer, and from 1.59 to 5.55 percent for the 6–12–6 fertilizer.

With 47 percent of soil moisture very little congestion of starch occurred in the fertilized plants as a result of difference in temperature.

With 56 percent of soil moisture, a 9° F. lower temperature (65° vs. 74°) caused a decided increase in starch (from 1.9 to 6.32 percent) with 6–12–6 fertilizer, but less increase with 12–0–12 and 12–6–12 fertilizer.

Variations in soil moisture also appeared to have a definite influence on the accumulation of starch. With temperature constant for unfertilized plants, and increase in soil moisture from 38 to 47 percent, there was an increase in starch from 1.27 to 9.4 percent; but with further increase in soil moisture to 56 percent the starch content dropped to 3.87 percent. Forty-seven percent of soil moisture was the best of the three moisture levels for starch congestion in the check plants under all three temperature conditions. Where fertilizers were used, there did not seem to be a definite soil-moisture optimum for starch accumulation. There was some tendency for this accumulation to be greatest at the 47-percent moisture level.

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STUDIES OF SELECTED STRAINS OF CURLY TOP VIRUS[1]

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INTRODUCTION

It has been known for some time[3] that the virus causing curly top shows wide variation in virulence as evidenced by symptoms produced on European brands of sugar beet (*Beta vulgaris* L.). Similar differences in severity of symptoms were also encountered in connection with greenhouse inoculations of susceptible sugar-beet varieties obtained from breeding work of this Division. These variations in severity of symptoms were usually attributed to lack of uniformity in resistance or susceptibility in the host plant or to attenuation of the virus. It was sometimes difficult to account for the variation on either of these assumptions, and results were slightly confusing. There has also been some disagreement among investigators with respect to the curly top susceptibility of certain varieties of sugar beet, bean, and other plants.

Studies made from 1932 to 1934 on the relative resistance to curly top of sugar-beet strains arising in the breeding work of this Division indicated that there is considerable variation in the reaction within a resistant strain or "variety." This variation was attributed to lack of genetic uniformity, since it was known that the breeding material tested had been developed largely by mass selection with little inbreeding. When geneticists of the Division obtained a sugar-beet line, designated as 1167, which was much more uniform as to curly top resistance, there was better opportunity for investigating the influence of the virus itself as related to variations in curly top reaction.

EXPERIMENTS WITH SUGAR BEETS

The new, highly resistant strain 1167 has been used as the resistant material in these studies. Some of the susceptible plants used were grown from a selected strain known as 2769–24 [4] and others were grown from a well-known European brand. There was little difference in the curly top reaction of the two susceptible strains and they are considered together.

Sugar-beet plants were inoculated in the cotyledon or young two-leaf stage, by placing one viruliferous beet leafhopper, *Eutettix tenellus* (Bak.), on each plant. The leafhoppers were allowed to remain on the plants for 1 week.

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[2] Acknowledgment is made to F. A. Abegg and F. V. Owen, of the Division of Sugar Plant Investigations, for constructive criticism and helpful suggestions, particularly in regard to statistical evaluation of experimental results.

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[4] 2769–24 designates a variety arising out of sugar-beet breeding work conducted by W. W. Tracy, Jr., of the Bureau of Plant Industry. Seed of this variety came as a result of direct increase of the original seed lot.

Plants were graded for severity of curly top symptoms according to a scale that ranged from 0 for no symptoms to 5 for the severest symptoms, as follows: Grade 1, either slight vein clearing or occasional papillate growths on the under side of the foliage, or both; grade 2, either slight curling of leaves with pronounced vein clearing or numerous papillae, or both; grade 3, pronounced curling and some dwarfing; grade 4, pronounced curling and increased dwarfing; grade 5, extreme curling and dwarfing. Illustrations of the type symptoms of these various grades of injury are given in plate 1 and figure 1.

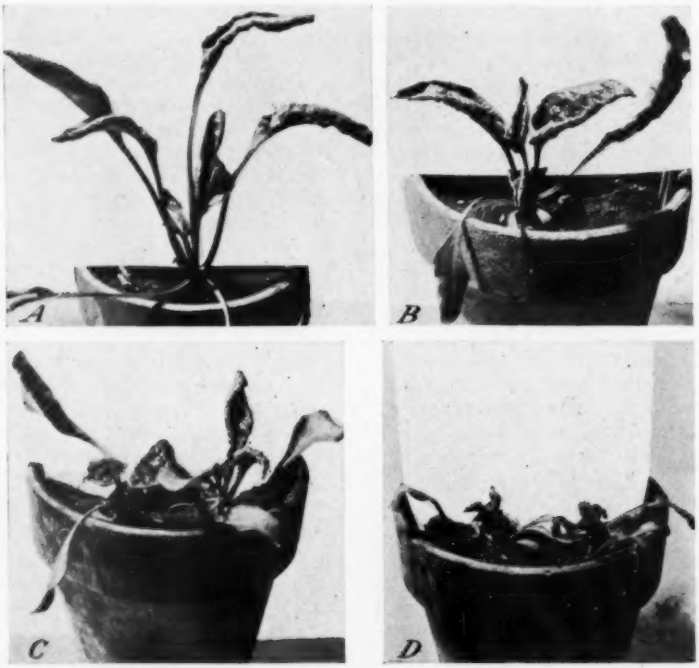
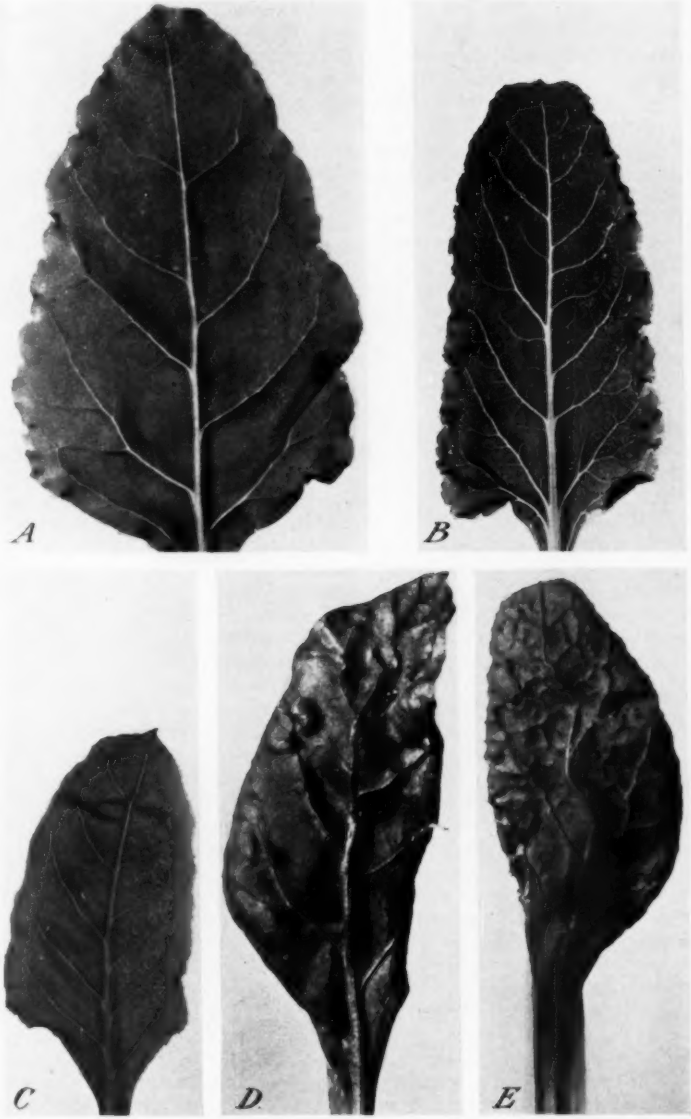


FIGURE 1.—*A*, Sugar-beet plant showing curly top symptoms that would be graded 2 in severity. There is usually pronounced vein clearing in the younger leaves, and numerous papillae, or wartlike growths, from the veins on the under side of the leaf. The leaves frequently show a tendency to roll or curl. *B*, Diseased sugar-beet plant showing pronounced leaf distortion and some dwarfing. This is classed as grade 3. *C*, Two sugar-beet plants much dwarfed and distorted by curly top. These symptoms are grade 4. *D*, Two sugar-beet plants showing extreme dwarfing and distortion. Such plants are likely to die in a short time, and the disease symptoms are recorded as grade 5.

Inoculation of resistant plants with virus from ordinary stock sources gave a wide variation in the percentage of plants infected, although the percentage was always significantly lower than for susceptible plants under similar conditions. The symptoms shown by resistant plants also differed widely, but, relatively, graded much lower than those on susceptible plants.

Some plants showing very mild symptoms and others with relatively severe symptoms were selected from the diseased resistant beets as virus sources for further inoculations, and similar selections were made from the diseased susceptible plants.



A, Normal, healthy sugar-beet leaf. *B–E*, Diseased sugar-beet leaves. *B*, Leaf of normal shape but showing vein clearing, most pronounced on right side of midvein. *C*, Slightly distorted leaf with vein clearing. Such vein clearing is quite characteristic of early symptoms in nearly all cases of curly top disease. In the very mild type of disease, there may be no other symptoms except possibly some small papillate outgrowths from the veins on the under side of the leaf. Plants showing symptoms to this extent, or less, are classed as grade 1. *D* and *E*, Diseased sugar-beet leaves showing large and much elongated papillate growths on under side of leaf; *D* might be graded 1 but *E* would grade 2.

Successive inoculations of the resistant and the susceptible test plants with virus from 94 such selected virus sources soon established the fact that the virus isolants could be classed in three major groups, as evidenced by distinctive reactions on the host plants. One or more plants from each group were selected as virus sources for further inoculations. For convenience in discussion, the virus isolants thus obtained will be referred to as virus strains, with the understanding that they may be further resolvable by appropriate technique.

Susceptible beets were readily infected by all three of the above virus strains, one inducing severe symptoms and the other two inducing mild symptoms. Resistant beets were quite easily infected by the strain that induced severe symptoms in the susceptible beets and by one of the two strains that induced mild symptoms in the susceptible beets, but they were quite resistant to infection by the other strain. Selections and inoculations have now been continued over a period of 2 years, and it has been found possible to differentiate clearly the symptoms induced by a fourth isolant.

In the earlier experiments, only three virus strains were included in each test and there was no replication as to virus source. In later work, the test of a virus strain was replicated by use of two or more different plants from a previous set of inoculations as independent virus sources. Each such replication is listed separately. Seven tests included all four virus strains at one inoculation, one test included only two, and the others included three virus strains variously chosen.

It seems that more reliable information is obtainable by consideration of all data for a strain as a whole, rather than by consideration of the separate comparative tests. The data as given in table 1 group plants inoculated at one time with a given strain. The uniformity of behavior is quite evident from the columns showing plants infected and grade of severity of symptoms. Considering the possibilities of variation in the plants, the environment, the virus, and the vector, the number of plants or experiments showing significant divergence from the general average is comparatively slight.

In the resistant beets, virus strain 1 shows 74 plants graded 3 and none graded 4 or 5; strain 2 shows only 5 plants graded 3 and none graded 4 or 5; and strains 3 and 4 show practically none above grade 1. In the susceptible beets, virus strains 1 and 3 show practically no plants in grades 1 or 2; strain 3 has relatively few in grade 3; and strains 2 and 4 have very few plants in grade 4 and none in grade 5.

The χ^2 test was used to determine statistically the relative significance of the differences between any one of these virus strains and each of the others. The computed χ^2 values are given in table 2.

The data from resistant beets, including all inoculated plants (grades 0 to 5, inclusive), show very highly significant differences among the virus strains. When severity of symptoms on infected plants only (grades 1 to 5, inclusive) is considered, the resistant beets show no significant difference between strains 3 and 4, but the differences between these two strains are brought out as highly significant by the susceptible beets in both the infected group and the group showing plants not infected. On the other hand, there are no significant differences between strains 2 and 4 in either group of susceptible plants, but highly significant differences between them do appear in the resistant groups.

TABLE 1.—*Frequency of occurrence of grades of severity of curly top symptoms in susceptible and in resistant sugar-beet plants when infected by virus strains 1, 2, 3, or 4, from July 1934 to February 1936, inclusive*

| Date | Number of resistant and susceptible plants showing indicated grade of severity of symptoms after inoculation with— | | |

| | | |

TABLE 2.—χ^2 *test as a means of estimating the significance of differences in curly top grade frequencies in sugar beets following inoculation of the test strain with various virus strains*

Range of curly top grades compared	χ^2 values from comparisons of virus strains—											
	1 and 2		1 and 3		1 and 4		2 and 3		2 and 4		3 and 4	
	Observed	Required [1]	Observed	Required [1]	Observed	Required [1]	Observed	Required [1]	Observed	Required [1]	Observed	Required [1]
RESISTANT BEETS												
1 to 5, inclusive	289.9	9.2	184.7	6.6	250.6	6.6	13.8	6.6	21.6	6.6	0.0	6.6
0 to 5, inclusive [2]	292.7	11.3	56.9	6.6	354.6	9.2	424.0	6.6	167.7	9.2	46.1	6.6
SUSCEPTIBLE BEETS												
1 to 5, inclusive	784.9	6.6	33.7	6.6	684.1	6.6	548.8	6.6	6.8	9.2	415.6	6.6
0 to 5, inclusive [2]	787.5	9.2	34.4	13.3	636.3	9.2	548.1	9.2	10.7	11.3	437.0	9.2

[1] For $P=0.01$.
[2] By including grade 0, the percentage of plants infected becomes a factor.

Table 3 presents a summary of the data from table 1. In this table the percentage of plants infected (ability of the virus to infect) is considered under the heading "Total plants," and the most valuable differential data for that group are in the percentage column of the resistant plants. The χ^2 test shows no significant difference between virus strains 1 and 2 in ability to infect resistant beets, but an extremely high significance between either 1 or 2 and 3 or 4. The difference between strains 3 and 4 is also highly significant. The virus strains show no significant differences in ability to infect the susceptible plants.

TABLE 3.—*Summary of percentages of infection and of curly top frequency grades, and average grade of disease severity in susceptible and in resistant sugar-beet plants when infected by different virus strains*

Virus strain No.	Tests	Total plants						Infected plants showing grade of severity of symptoms [1]										Average grade of severity for diseased plants	
		Resistant			Susceptible			Resistant					Susceptible					Resistant	Susceptible
		Inoculated	Infected		Inoculated	Infected		1	2	3	4	5	1	2	3	4	5		
	No.	*No.*	*No.*	*Pct.*	*No.*	*No.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*		
1	51	1,067	683	64	893	708	79	18	71	11	0	0	0	2	16	39	43	1.9	4.3
2	29	592	360	61	527	423	80	72	26	1	0	0	7	75	17	1	0	1.3	2.1
3	33	773	67	9	505	409	81	94	3	3	0	0	0	1	8	31	60	1.1	4.5
4	21	454	103	23	365	275	75	94	6	0	0	0	2	76	21	1	0	1.1	2.2

[1] Percentages are based in each case on the total number of diseased plants among those that had been inoculated with the specified virus strain.

In any comparative study of differential reaction to the curly top virus, severity of symptoms is an entirely different criterion from percentage of infection, and in table 3 the diseased plants are considered

as a distinct group under the heading "Infected plants." The percentages given here are based on the total number of diseased plants among those inoculated with the specified virus strains. Considering the resistant beets, virus strain 1 was the only strain inducing grade 3 symptoms in an appreciable percentage of plants, and its ability to infect was the highest of any; strain 3 showed very low ability to infect (9 percent as compared with 64 percent for strain 1), and 94 percent of all infected plants graded 1 in severity. Virus strain 1 also induced severe symptoms on susceptible beets, but strain 3 gave a significantly higher percentage in the grade 5 class and a higher average severity of symptoms. From all data on resistant beets, virus strain 1 would be classed as far more virulent than strain 3; while the data on susceptible beets would indicate strain 3 as the more virulent.

All four virus strains produced high percentages of infection in susceptible beet varieties. Strain 1 induced severe symptoms in the susceptible variety and a high percentage of infection with mild to intermediate severity in the resistant variety. Strain 2 induced mild symptoms in the susceptible variety and a relatively high percentage of infection but very mild symptoms in the resistant variety. Strain 3 produced extremely severe symptoms in the susceptible plants but rarely infected a plant of the resistant strain, and any resistant beets infected developed very mild symptoms. Strain 4 gave mild symptoms in the susceptible variety and a small percentage of infection with very mild symptoms in the resistant variety.

The four virus strains may be roughly grouped according to the plan given in table 4, in which plus signs (+) indicate high percentages of infection or relatively severe symptoms and minus signs (−) indicate low percentages of infection or relatively mild symptoms. This grouping is based upon data from a large number of comparative inoculation experiments. The smallest number of plants involved was 365 in the case of susceptible plants tested with virus strain 4, while the largest number was 1,067 of the resistant plants tested with virus strain 1.

TABLE 4.—*Classification of virus strains according to ability to infect and severity of symptoms in sugar beets* [1]

Virus strain No.	Relative percentage of infection—		Relative severity of symptoms in—	
	Susceptible beets	Resistant beets	Susceptible beets	Resistant beets
1	+	+	+	+
2	+	+	−	−
3	+	−	+	−
4	+	−	−	−

[1] Plus signs (+) indicate high percentages of infection or relatively severe symptoms; minus signs (−) indicate low percentages of infection or relatively mild symptoms.

The percentages of plants showing different grades of severity of symptoms are presented graphically in figure 2.

Virus strains 1, 2, and 4 were readily maintained as "cultures" in either the susceptible or the resistant beets, but some difficulty was

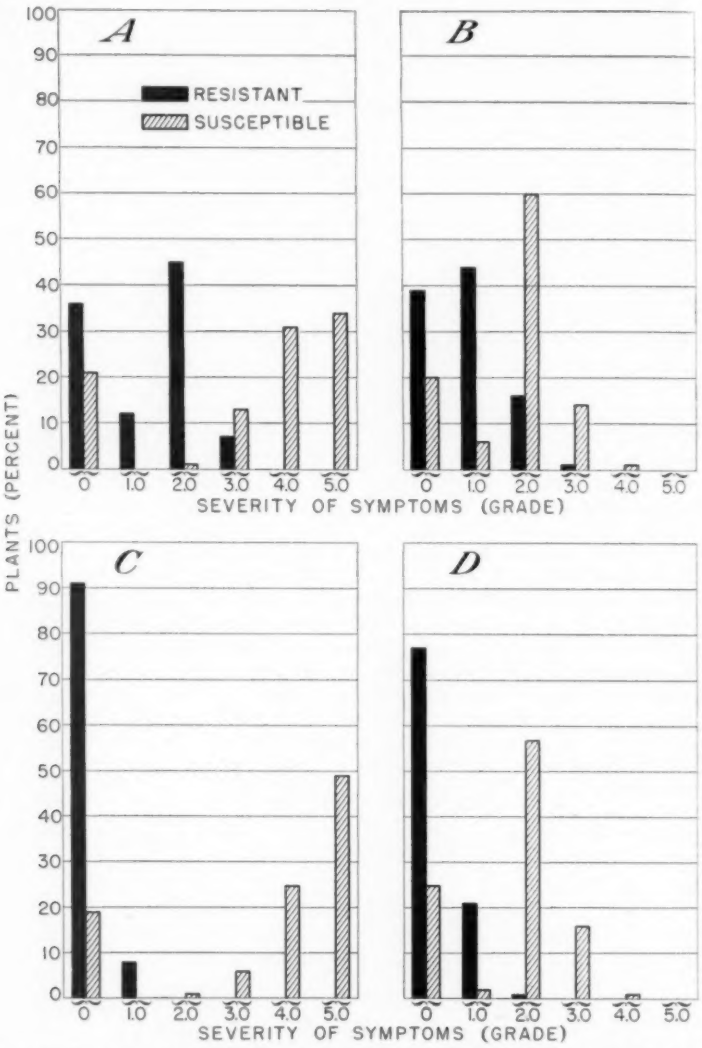


FIGURE 2.—Percentages of sugar-beet plants showing different grades of severity of symptoms after inoculation with different curly top virus strains. (0=no infection.) *A*, Virus strain 1; *B*, virus strain 2; *C*, virus strain 3; *D*, virus strain 4.

experienced in the case of strain 3 because the young susceptible plants were often so severely injured that they died and very few of the resistant plants became infected. It was also found that virus strain 3, when obtained from resistant beets, frequently had undergone a change in virulence which appeared to be permanent. Further studies regarding virus changes, with possible relation to virus strains, are in progress.

REACTIONS OF OTHER HOSTS TO THE VIRUS STRAINS

These four strains of curly top virus have been tested on tomato *Lycopersicon esculentum* Mill.), tobacco (*Nicotiana tabacum* L.), bean (*Phaseolus vulgaris* L.), plantain (*Plantago erecta* Morris), and peppergrass (*Lepidium nitidum* Nutt.)

It may be noted that the numerous hairs on tomato, tobacco, and bean seem to entangle and irritate the leafhoppers, resulting in their early death. Scraping the leaves gently with a safety-razor blade or rubbing them with cloth to remove many of the hairs resulted in a greatly decreased mortality. Unless otherwise noted, four leafhoppers per plant were used on tomato, bean, and tobacco, and one leafhopper per plant on plantain and peppergrass. The leafhoppers were allowed to remain on the plants for a week.

The Stone variety of tomato was used, and the plants were inoculated when they were 3 to 4 inches tall. The results are given in table 5. Virus strains 1 and 3 gave fairly high percentages of infection, while strains 2 and 4 gave no infection.

Inoculations into Turkish tobacco furnished data that are likewise presented in table 5. The plants were inoculated when they were 2 to 6 inches tall. Four leafhoppers per plant were used for the January inoculation, and eight per plant for the March and May inoculations. The greater effectiveness of the larger number of insects is clearly apparent. Percentages of infected plants in this host closely parallel those for tomato. Neither virus strain 2 nor strain 4 produced any symptoms on tobacco, and numerous attempts to recover the virus from such inoculated plants gave negative results.

Three varieties of bean were used, and inoculations were made before the second leaf had opened out. The Red Mexican variety was selected for extremely high resistance, and there was no evidence of infection on any of the inoculated plants. Attempts to recover the virus from these plants gave negative results.

Two selected strains of Great Northern, namely, the Ellsworth strain and the University of Idaho No. 81 strain, were selected for intermediate resistance. There was no evidence of any significant difference in the reactions of these two strains of bean, and the results from both are included in table 5. Virus strain 4 gave a small amount of infection in these beans, while strain 2 gave no infection. The Great Northern bean variety, the plantain, and the peppergrass are the only hosts so far tested, except beet, that have served to differentiate virus strains 2 and 4.

The results of inoculation of Bountiful beans are given in table 5. There was a high percentage of infection with each of the four strains of virus, but the symptoms induced by strains 1 and 3 were markedly more severe than those induced by strains 2 and 4. Infection by strains 1 and 3 resulted in early loss of leaves, complete cessation of growth, and relatively early death.

The plantain (*Plantago erecta*) plays a very important role in the life of the sugar-beet leafhopper under California conditions, and its reaction to the different virus strains is of particular interest. The plants were inoculated in the two- to four-leaf stage. Difficulty has been experienced in growing this plantain under greenhouse conditions and in being certain as to symptoms. It seems necessary to rely upon curling and twisting of the leaves as symptoms in both the plantain

and the peppergrass. The severe cases are easily noted, unless the plant dies without making appreciable growth after inoculation, but slight curling or twisting may be confused with some other type of injury or with normal growth changes. Table 5 gives the data thus far secured.

TABLE 5.—*Effect on various hosts of inoculations with different strains of virus of curly top of sugar beet*

Variety and date	Strain 1			Strain 2			Strain 3			Strain 4		
	Plants inoculated	Plants infected		Plants inoculated	Plants infected		Plants inoculated	Plants infected		Plants inoculated	Plants infected	
	Number	*Number*	*Percent*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
TOMATO												
Stone:												
June 20, 1935	60	26	43	60	0	0	40	19	48	20	0	0
July 26, 1935	112	82	73	80	0	0	40	19	48	20	0	0
May 28, 1936	36	11	31	16	0	0	52	18	35	17	0	0
Total	208	119	57	156	0	0	132	56	42	57	0	0
TURKISH TOBACCO												
Jan. 17, 1936	19	7	37	19	0	0	57	18	32	19	0	0
Mar. 3, 1936	71	50	70	20	0	0	53	31	58	12	0	0
May 28, 1936	40	33	83	8	0	0	60	44	73	20	0	0
Total	130	90	69	47	0	0	170	93	55	51	0	0
BEAN												
Great Northern:												
July 27, 1935	65	28	43	34	0	0	33	4	12	10	1	10
Sept. 10, 1935	60	18	30	24	0	0	57	27	47	2	1	50
July 11, 1936	76	16	21	0	0	0	341	184	54	36	3	8
Total	201	62	31	58	0	0	432	215	50	48	5	10
Bountiful:												
July 27, 1935	58	[1] 43	74	32	21	66	32	[1] 28	88	8	8	100
PLANTAIN												
Nov. 27, 1935	11	6	55	1	0	0	11	8	73	10	1	10
Feb. 7, 1936	54	32	59	29	0	0	43	35	81	8	0	0
Mar. 23, 1936	189	135	71	76	0	0	199	124	62	56	2	4
May 1, 1936	65	54	83	32	0	0	90	87	87	33	0	0
Total	319	227	71	138	0	0	352	254	72	107	3	3
PEPPERGRASS												
Nov. 27, 1935	112	50	45	41	0	0	74	65	88	38	1	3
Feb. 7, 1936	87	54	62	46	0	0	69	64	93	20	1	5
May 1, 1936	55	41	75	27	0	0	81	68	84	26	2	8
Total	254	145	57	114	0	0	224	197	88	84	4	5

[1] About 70 percent of the Bountiful bean plants infected with virus strains 1 or 3 lost their terminal buds and ceased all growth in less than 3 weeks after inoculation.

Some infection was obtained with virus strain 4 but none with strain 2, while relatively high infection percentages were secured with strains 1 and 3.

To study these strains of virus in *Plantago* under more nearly natural conditions, inoculations were made upon plants grown in cages in the field. The plantings were made in January, which was rather late for satisfactory results. Only three virus strains (1, 3, and 4) were used. The plants were grown under cloth-covered cages 18 inches square. When the plants were 6 weeks old, those in each cage were inoculated with one of the three virus strains used. These plants were tested in May, and each strain was recovered from the group that had been inoculated with it.

The native peppergrass is also an important California host of the leafhopper. Inoculation results (table 5) were very similar to those from plantain. The percentage of plants infected with strain 4 was very low, and strain 3 gave a much higher rate of infection than strain 1. Because of the occasional symptoms produced in plants inoculated with virus strain 4, it seemed desirable to test a number of the plants inoculated with strains 2 and 4. Nineteen plants inoculated with strain 4 and 28 plants inoculated with strain 2 were tested by placing nonviruliferous leafhoppers on them and then transferring these leafhoppers to susceptible beets. In no case was there evidence of beet infection unless the peppergrass had been graded as diseased.

DISCUSSION

It seems clearly demonstrated that curly top virus is a complex of strains that can be separated into recognizable entities by differential host-plant responses. In this study four such strains have already been differentiated, and, considering the limited number of hosts used and virus sources tested, it appears certain that many other curly top virus strains will eventually be recognized. The existence of strains within the curly top virus may have a significant relation to breeding which seeks to develop further, in sugar beet or in other plants, varieties resistant to curly top. It is conceivable that a virus strain which had not previously been a factor might suddenly find favorable conditions for widespread development with more or less disastrous results. There are some sugar-beet varieties and many varieties of other economically important crop plants that have not been tested with the four virus strains described in this paper. Their response to inoculation with any of these four virus strains cannot be predicted.

In field studies or in commercial sugar-beet fields, the prevalence of one or another strain of virus might result in quite different symptoms and injury. It is highly important to learn the factors that influence the predominance of any strain of virus in certain areas or during certain seasons. Such data are being sought by field and greenhouse studies of the virus strains in relation to native and overwintering curly top host plants.

These studies furnish at least a partial explanation of the inconsistent or divergent results occasionally secured in curly top studies. An investigator testing virus strain 3 on resistant sugar beet 1167 would certainly conclude that this variety is practically immune to curly top, but if the inoculum happened to be strain 1 this resistant variety would be rated as quite susceptible to infection and subject to appreciable injury. Similar discrepancies might easily occur as a result of work with any of the strains described.

The more virulent virus strains, 1 and 3, induce extremely severe symptoms on the most important overwintering hosts, *Lepidium*, *Plantago*, and filaree. The young plants are often killed or reduced to a very small, inconspicuous tuft of growth. This fact suggests that these highly virulent strains tend to be self-limiting under certain conditions and it may help to explain the greater prevalence of the less virulent strains during some seasons or in some areas.

CONCLUSIONS AND SUMMARY

Four curly top virus strains have been recognized by the differential reactions of sugar beets, percentages of plants infected and severity of symptoms being used as the bases of comparison. Beets of the resistant line 1167 showed distinctive reactions to each of the four virus strains, and the susceptible beets gave reactions that differentiated strain 1 from strain 2, 1 from 3, 1 from 4, 2 from 3, and 3 from 4. Virus strains 2 and 4 could not be separated by the responses of the susceptible test beets.

In susceptible beets, virus strains 1 and 3 induced severe symptoms, whereas strains 2 and 4 produced only mild symptoms. The percentages of susceptible plants infected were approximately the same for all four virus strains.

In beets of the resistant line 1167, virus strain 1 induced obvious (not severe) symptoms, but strains 2, 3, and 4 produced only inconspicuous symptoms. The percentages of resistant beets infected by virus strains 1 and 2 were high, whereas for strains 3 and 4 they were very low.

The more virulent strains, 1 and 3, were easily distinguished from the less virulent strains, 2 and 4, by inoculation into tobacco or tomato; but these hosts did not differentiate strain 1 from 3 or 2 from 4.

The bean varieties, as well as *Plantago erecta* and *Lepidium nitidum*, did not give responses that were satisfactory for distinguishing strain 1 from strain 3, although *Lepidium* gave a significant difference between strains 1 and 3 in the percentages of plants infected.

These experiments show that the curly top virus exists as strains which vary in ability to infect and injure different hosts.

MOSAIC OF LIMA BEANS (PHASEOLUS LUNATUS MACROCARPUS) [1]

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INTRODUCTION

In 1936 Harter (*4*)[2] published an abstract calling attention to a mosaic of lima beans (*Phaseolus lunatus macrocarpus* Benth.)[3] that appeared in Maryland in the summer of 1935. It was stated therein that, while the symptoms of the disease somewhat resembled those of the common bean mosaic, it could not be transmitted to beans (*P. vulgaris* L.). For this and other reasons, it was believed to be different from bean mosaic and from the mosaics of other legumes. Certain symptoms obtained from inoculating the lima bean mosaic virus into tobacco (*Nicotiana tabacum* L.), cucumber (*Cucumis sativus* L.), and broadbean (*Vicia faba* L.) suggested its possible identity with or close relationship to the cucumber mosaic virus.

Specimens of mosaic-infected lima beans were collected in the breeding grounds of the Division of Fruit and Vegetable Crops and Diseases located near Beltsville, Md., from a mixed population of hybrid progenies of lima beans. Five to ten percent of the plants were diseased and were scattered more or less indiscriminately over the field. The first symptoms appeared in a mild form in July 1935. The number of diseased plants and the severity of the symptoms increased as the season advanced. A detailed study of lima bean mosaic was begun in September of the same year, when a number of infected plants were removed from the field to the greenhouse to provide a source of mosaic material for further study. These plants flourished under greenhouse conditions and in a few weeks produced an abundance of foliage showing the characteristic field symptoms of mosaic.

The same symptoms were observed again in 1936 in the lima bean breeding trials, although a number of the more susceptible progenies had not been planted.

DISTRIBUTION AND ECONOMIC IMPORTANCE

Lima bean mosaic has been reported from widely separated sections of the country. McClintock (*6*) reported its occurrence on several varieties of lima beans of the sieva type at Norfolk, Va., in 1916. It was not found on any of the large-seeded Fordhook types. He concluded that, since lima beans had not been grown on the land previous to 1916, the infection was carried by the seed. Adams (*1*) briefly stated that a lima bean mosaic was generally distributed in Delaware in 1923 and that it was of increasing importance in that State. Osner

[1] Received for publication September 2, 1937; issued June, 1938.

[2] Reference is made by number (italic) to Literature Cited, p. 905.

[3] In ordinary botanical usage, *Phaseolus lunatus* is the civet or sieva bean and *P. lunatus macrocarpus* the lima bean.

(*8*) listed it among the diseases of economic importance in Indiana in 1917, and Harrison [4] reported its occurrence on several varieties in New York in 1936. The writer observed the mosaic on several varieties in Michigan in 1926, and McCubbin has reported (though not published) its occurrence on the Challenger lima in Cuba. Cook (*2*) listed the lima bean among the crops of Puerto Rico attacked by a virus which he suspected might be the same as the bean mosaic. It was not seed-borne, and *Aphis rumicis* L. was thought to be a possible vector. These reports of the wide geographical distribution of the disease indicate that its presence in any part of the country should occasion no surprise.

No estimates are available as to the losses caused by the lima bean mosaic. Reports of its increasing economic importance and general observations indicate that it is on the increase and may eventually become a serious menace to the crop if susceptible varieties are more widely grown.

MATERIALS AND METHODS

SOURCE OF VIRUSES

The lima bean virus used in these experiments was obtained originally from field plantings near Beltsville, Md., or from plants grown in the greenhouse, where conditions could be controlled and the plants protected against contamination by insects carrying the virus from other susceptible hosts. The virus is very potent in the leaves, and careful comparison of infected field- and greenhouse-grown plants showed the symptoms to be identical.

Because of the close similarity of the lima bean mosaic to the mosaic of cucumbers (*3*), the two causal viruses have been carefully compared.[5] To do this cross inoculations were made and a number of hosts susceptible to the cucumber mosaic were inoculated with the virus from lima beans.

METHODS

The method of inoculation employed was the one generally used in the mechanical transmission of viruses and was briefly as follows: A supply of viruliferous material was maintained in the greenhouse by inoculation of lima beans and Turkish tobacco (*Nicotiana tabacum*). The inoculum was taken from the leaves of one or the other of these hosts. The leaves were thoroughly macerated and the juice was squeezed out through cheesecloth. To the liquid was added about 2 volumes of water to 1 of plant juice. The ratio of water to plant juice is not important, provided the dilution is not too great. Preparatory to inoculation, the leaves were dusted with a small quantity of finely pulverized carborundum powder. The inoculations were made by rubbing the upper side of the primary leaves with a small quantity of absorbent cotton wrapped in cheesecloth that had been dipped in the inoculum. The best results are obtained by rubbing only sufficiently hard to produce slight abrasions of the leaf. A few minutes after the leaves were rubbed, most of the inoculum was removed by spraying the leaves with a small amount of water. These methods have been

[4] HARRISON, A. L. VARIETAL SUSCEPTIBILITY OF LIMA BEANS TO MOSAIC. U. S. Bur. Plant Indus. Plant Disease Reptr. 20: 291. 1936. [Mimeographed.]

[5] The cucumber virus and the celery virus, which it closely resembles and which was used to a limited extent in a comparative study, were supplied by S. F. Doolittle, of the Division of Fruit and Vegetable Crops and Diseases.

followed more or less closely throughout these investigations and have given highly satisfactory results.

HOST RANGE

Hundreds of lima beans, including most of the different varieties, and a considerable number of other plants have been inoculated with the lima bean mosaic virus. A sufficient number of different genera were employed to show that the lima bean mosaic virus will infect hosts widely separated in relationship. Because of the apparently close relationship of the lima bean mosaic to the cucumber mosaic, some of the plants known to be susceptible to the latter were used for comparison. The results of the host-range studies appear in the following tabulation:

Plants inoculated:	Reaction [1]	Plants inoculated—Cont'd.	Reaction [1]
Cucumis sativus L.	+	*Datura stramonium* L.	−
Nicotiana tabacum L. (Turkish)	+	*Polygonum aviculare* L.	−
Phaseolus vulgaris L. (Refuge)	−	*Petunia hybrida* Vilm.	+
Zinnia elegans Jacq. (Double Fantasy)	+	*Vicia faba* L. (Broadbean)	+
Lycopersicon esculentum Mill. (Globe)	+	*Vigna sinensis* Torner (Black and Blackeye)	+
Pisum sativum L. (Harrisons Glory)	−	*Trifolium hybridum* L.	−
Lathyrus odoratus L.	−	*Vicia sativa* L.	−
Phytolacca americana L.	+	*Vicia villosa* Roth	−
Solanum melongena L. (Superior High Bush)	+	*Lens esculenta* Moench	−
Capsicum annuum L. (World Beater)	+	*Phaseolus calcaratus* Roxb.	−
		Lupinus albus L.	−
		Soja max (L.) Piper	−
		Zea mays L.	−
		Zea mays everta Bailey	+

[1] Minus signs (−) indicate resistance; plus signs (+), susceptibility.

SYMPTOMATOLOGY

LIMA BEAN

The symptoms of the lima bean mosaic can be described best by tracing the changes that take place in the plant, especially in the leaves, from the first visible symptoms progressively through the different stages of growth to the mature leaves.

Similar to the mosaics of the many other hosts, the earliest symptoms on lima bean are characterized by a slight clearing of the veins of the juvenile leaves, which is soon followed by some very striking leaf symptoms. The lamina of the leaflet may bend downward to an angle of 45° or more, accompanied at the same time by a recurving downward of the midrib. Frequently, the plant dies in this stage. If, however, the attack is not severe enough to kill the plant, the leaflets and the midribs in the processes of growth gradually resume their normal position. At this time or when the leaflets and midribs are still curved downward, the vine grows from 1 to 2 inches long. The apical end of the vine often curves downward instead of assuming the erect position of a normal plant. The second pair of unfolding leaflets hang limply from the pulvinus.

Inoculations were made by rubbing inoculum into the primary leaves at about the time they were fully grown or 1 or 2 days earlier; i. e., just before any vine growth had started. Under the most favorable conditions, symptoms appear in 5 to 6 days. If the plant survives the first onslaught of the disease, vine growth starts and a full-sized

plant may eventually result although the rate of growth is considerably retarded. The drooping leaves slowly return to their normal position, and a slight yellow flecking may be observed at about this time (pl. 1, *A*). Concomitantly with the further growth of the plant the flecks on the leaves increase in number and size (pl. 1, *B* and *C*). Sometimes, instead of flecks, larger light-colored areas of various sizes occur, often, though not always, near the margin (pl. 1, *D* and *E*). By the time the leaves reach maturity the flecks combine to form alternate light and dark spots characteristic of mosaic in many other plants. Irregularities in the growth of different portions of the leaf, or other causes, result in the production of islands of raised or depressed spots (pl. 1, *F* and *G*), of various sizes, composed of dark-green tissue. The entire series of changes and symptoms is completed by the time the host has developed the third or fourth set of trifoliate leaves.

Occasionally primary lesions occur on the inoculated primary leaves. They are characterized by a clearing of the veins and veinlets. This is accompanied by the development of spots, in the form of a distinct yellow mottling, that are distributed more or less uniformly over the entire leaf. The spots are indeterminate in outline and bear no direct relation to the veins. The evidence of primary leaf infection occurs in about 5 days after inoculation.

The lima bean virus has not been found to produce any symptoms on the pods.

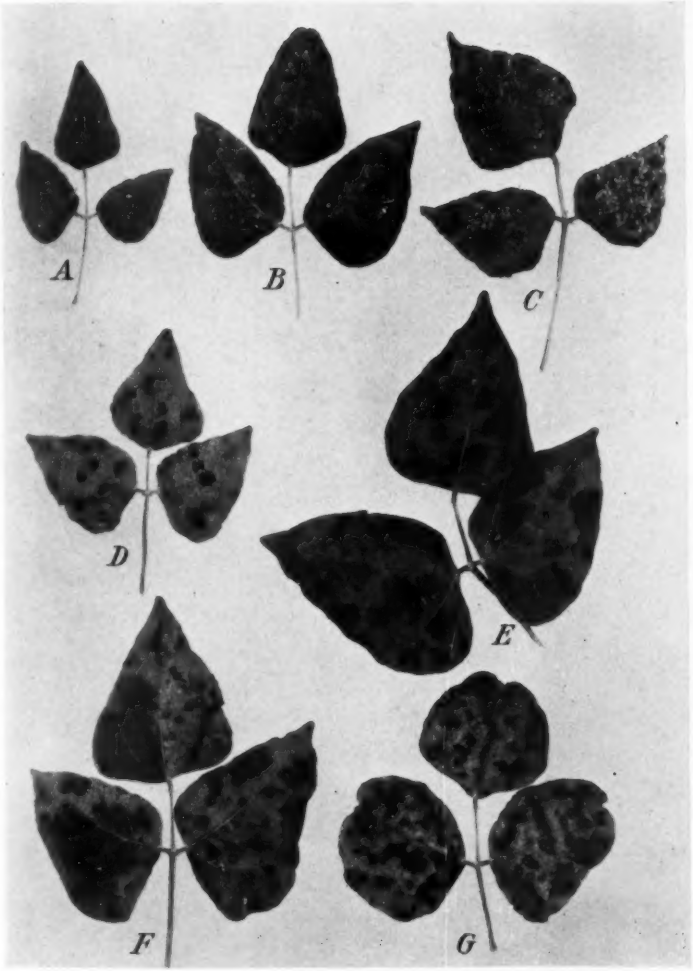
COWPEA

Only local lesions are produced by the lima bean virus on the Black and on the Blackeye cowpea (*Vigna sinensis*). These lesions are visible in 48 hours, and under favorable conditions (80°–100° C.) in 36 to 40 hours. They appear as minute specks (pl. 2, *I*) that gradually enlarge and become somewhat irregular in outline. When fully developed they range from 0.5 to 3.5 mm in diameter. These specks are purplish in color and sometimes are surrounded by a narrow band of light-green tissue. The centers of the spots are sometimes gray in color. Local lesions may be produced on the trifoliate leaves, though they develop there somewhat more slowly than on the primary leaves.

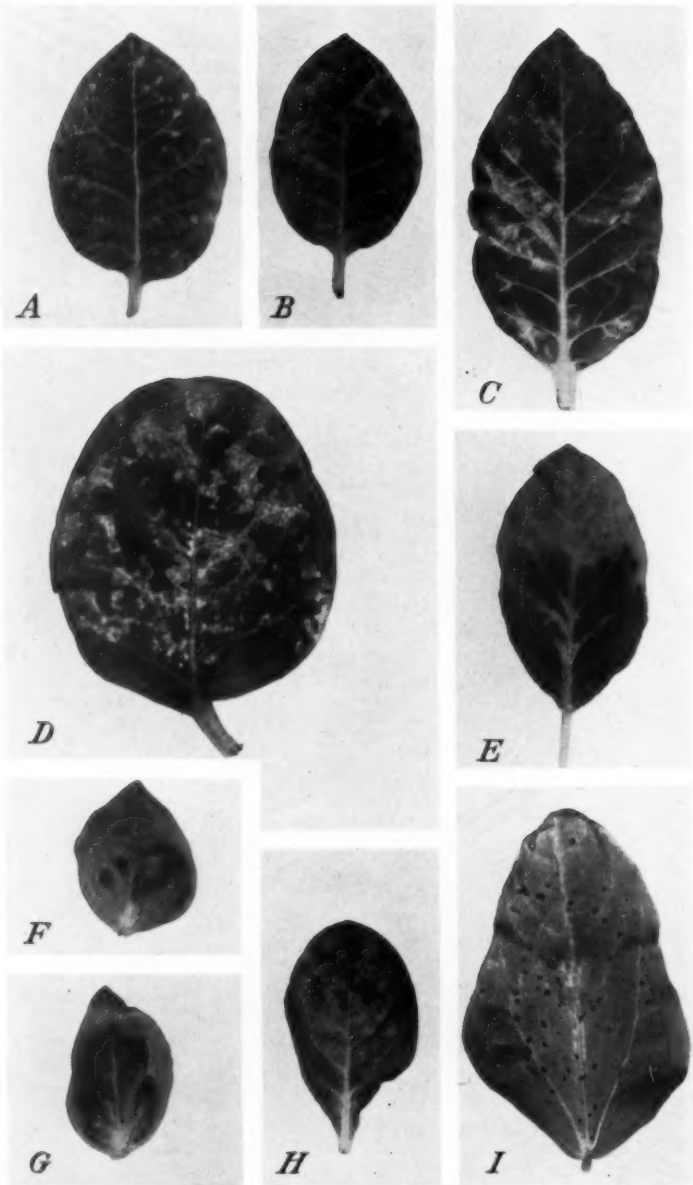
The local lesions on the Black and on the Blackeye cowpea, which for the most part are very similar, differ in one or two details. The Black cowpea lesions continue to increase in size for several days or until they attain a diameter of about 3.5 mm, whereas the lesions on the Blackeye cowpea remain small, usually not exceeding 1 or 2 mm in diameter. The difference in the size of the lesions is very consistent and apparently is strictly a varietal characteristic.

TOBACCO

The lima bean virus on tobacco (*Nicotiana tabacum*) causes symptoms that are characterized by retarded growth and a clearing of the veins, accompanied by a slight yellowing of the young leaves 5 to 7 days after inoculation. These early symptoms are frequently followed by the development of small yellowish spots (pl. 2, *A*) arranged more or less uniformly over the entire leaf. The spots gradually enlarge, some coalescing with others to form areas of various sizes and shapes (pl. 2, *B*). No further changes occur on these leaves but those subsequently developed may acquire two distinct patterns, one in which there is a yellowing in large localized areas, circumscribed to some extent by the large lateral veins (pl. 2, *C*), and the other in which



A–G, Stages in the development of lima bean mosaic on lima beans.



A, B, C, E, Stages in systemic infection of *Nicotiana tabacum* by lima bean virus; *D,* primary infection, showing different patterns on inoculated leaf of *Nicotiana tabacum;* *F, G, H,* stages and symptoms of infection by lima bean virus on leaves of *Petunia hybrida; H,* first visible symptoms; *I,* three-day-old local lesions on leaves of *Vigna sinensis* (Black variety).

large portions of the tip (pl. 2, *E*) or other parts of the leaf may be involved irrespective of the veins. These symptoms are not very different, if at all, from those caused by the cucumber virus.

Primary lesions generally result on the inoculated leaf of tobacco. These lesions are distributed more or less evenly over the leaf and vary greatly in form and size. Rings or circles of light-green tissue sometimes form a portion of the mosaic pattern. The circular and other spots and patterns are outlined by finely etched lines that differ markedly in general appearance from the mosaic symptoms on other leaves of the plant. The assortment of patterns is illustrated by plate 2, *D*.

PETUNIA

The leaves of young plants of petunia (*Petunia hybrida*) inoculated with the lima bean virus are slow to develop characteristic symptoms. Some stunting is evident in about 10 days, and soon thereafter the leaves show an unhealthy condition. Light-yellowish spots (pl. 2, *H*), indistinct at first, appear distributed more or less uniformly over the entire leaf.

When the plants become older, the characteristic symptoms are very much changed. The mild mottling of young plants is replaced on the leaves of older ones by islands of badly malformed tissue, in which apparently unequal growth of cells results in the production of dark-green blisterlike spots of various sizes. These malformed areas do not usually extend to the margin (pl. 2, *F* and *G*) of the leaf, although a considerable portion of the leaf may be affected. The leaves are much smaller than those on normal plants of the same age.

PEPPER

Pepper (*Capsicum annuum*), when inoculated with the lima bean virus, does not always give consistent or uniform symptoms. A distinct retardation of growth is the first evidence of infection and is noticeable in from 10 to 14 days after inoculation, followed soon thereafter by a slight clearing of the veins. The basal portion of the leaf is more or less uniformly yellowed (pl. 3, *A*), although sometimes yellowish irregularly shaped spots, distributed over the leaf and between the veins, are developed instead. These spots resemble typical mottling, and can be seen to the best advantage by transmitted light. The yellow color of infected leaves gradually increases in intensity with the increased age of the plant. A slight copper color may replace some of the yellow in old leaves. In the late stages of the disease, the veinlets are perceptibly cleared, giving the leaf somewhat the appearance of network.

TOMATO

The tomato (*Lycopersicon esculentum*) plant is badly stunted and malformed when infected by the lima bean virus (pl. 3, *D*). The mosaic symptoms appear in about 10 days after inoculation. The leaves are mottled, and the new leaf and stem growth is decidedly filiform. The plants frequently die.

CUCUMBER

Young cucumber plants (*Cucumis sativus*), when inoculated with the lima bean virus, show symptoms in about 6 to 10 days. Growth is retarded, and there is a decided clearing of the veins of the leaves.

The leaves are yellowed, the margins curl slightly upward, and as the plant becomes older a glossy appearance develops on the stem from the leaves to the roots. Plants so affected die within a few days. The symptoms are very similar to if not identical with those caused by the cucumber virus.

ZINNIA

The lima bean virus on zinnia (*Zinnia elegans*) does not cause much if any stunting of the plant. The symptoms appear in from 5 to 10 days after inoculation. Soon thereafter mottling occurs on the leaves above the inoculated pair, the spots becoming more distinct with the increasing age of the plant. In the late stages of development, the light and dark spots are sharply defined. The apex of the leaf in most cases becomes uniformly yellowed and very often growth of that part of the leaf is retarded, causing some malformation (pl. 3, *F*). Mottling occurs on the petals and on the leaves of branches developed below the inoculated leaves.

POKEWEED

Pokeweed (*Phytolacca americana*), like zinnia, is little injured by the lima bean virus. In about 2 weeks after inoculation light-yellow spots (pl. 3, *C*) appear scattered irregularly over the surface of the leaf. These spots have no definite outline and bear no order or arrangement in relation to the midrib and veins of the leaf.

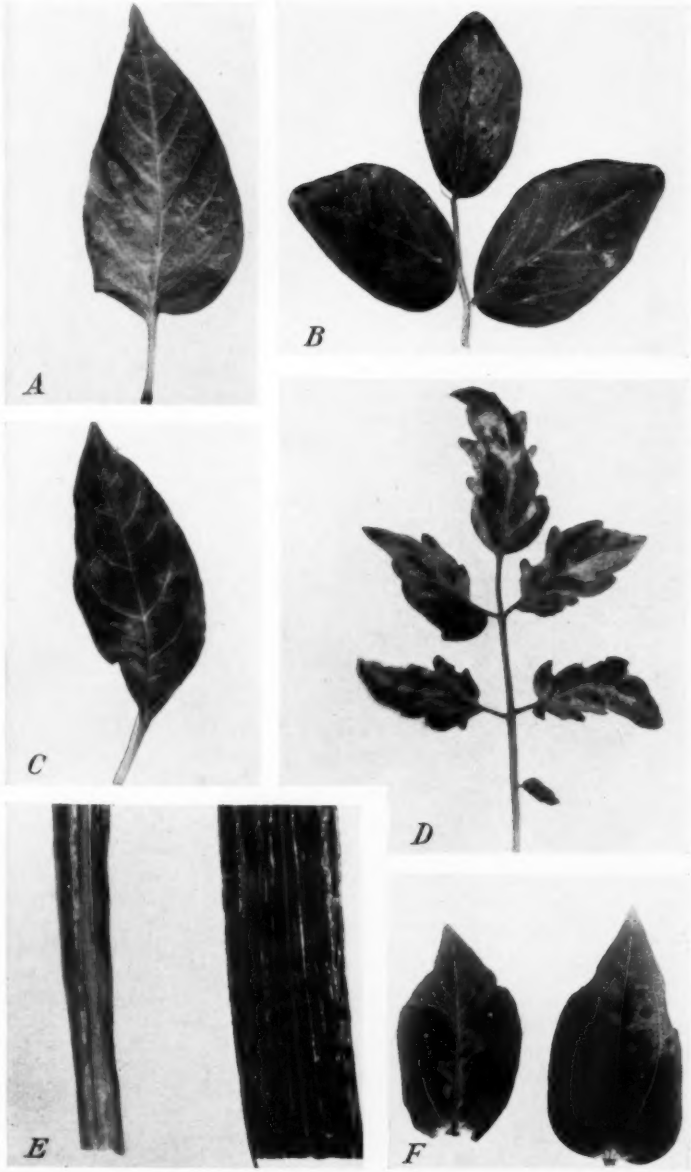
BROADBEAN

The broadbean (*Vicia faba*), although susceptible to the lima bean virus, has never given any consistent results in inoculation tests. Sometimes typical local lesions are produced, and occasionally systemic infection has been obtained. If the inoculum is taken directly from the lima bean plant, no local lesions, or only a very few, develop. If, however, the inoculum is derived from the Turkish tobacco, typical local lesions (pl. 3, *B*) appear in about 4 or 5 days. These lesions are somewhat purplish, and while not so numerous as those on cowpea, they closely resemble them. What the difference in the viruses consists in is not certain, but it has been suggested that the virus is more potent when taken from tobacco than when derived from lima bean.

Systemic infection occurs in a very small percentage of inoculations. The leaves are mottled, but little if any dwarfing of the plant results. When inoculated back to lima beans, the normal lima bean mosaic symptoms are always obtained.

CORN

Wellman (*11*) showed that many plants of the Gramineae, among them corn (*Zea mays*), were readily infected when inoculated with the celery mosaic virus. Price (*9*) proved later that the cucumber virus would also infect corn. The writer has inoculated *Zea mays* with the lima bean virus and has obtained only very mild symptoms in the form of faint streaks characterized by a clearing of the veins. While the infection becomes systemic, no injury to the plant or retardation of growth results. If, on the other hand, popcorn (*Z. mays everta*) is inoculated with the same virus very different results are obtained. In about 7 days after inoculation some yellowing of the leaves occurs, but the most striking symptoms are long alternate green and yellow



Symptoms of lima bean mosaic on various hosts: *A*, Vein clearing and yellowing of leaves of *Capsicum annuum;* *B*, local lesions on leaves of *Vicia faba;* *C*, mottling of leaves of *Phytolacca americana;* *D*, malformation of leaf of *Lycopersicon esculentum;* *E*, yellow streaks and spots on leaves of *Zea mays everta* (popcorn); *F*, leaves of *Zinnia elegans* showing yellow color and retarded growth of apical ends.

streaks (pl. 3, *E*) on the leaves. Later, islands of yellow tissue develop outside or between the vascular bundles. These partly chlorotic areas vary greatly in size and form. The disease increases in severity with the increase in age of the plant. The symptoms become more conspicuous with each new leaf developed until finally almost the entire leaf is uniformly yellowed. In the later stages of the most severe cases the margins of the leaves and the tips of young leaves die and turn brown.

VARIETAL RESISTANCE AND SUSCEPTIBILITY

No attempt has been made to collect all the different varieties of lima beans. Those that have been included in the experiments were obtained from several seed companies. The question of synonymy has not been taken into account, each seed lot being carried under the name given it by the contributor.

The results of identical inoculations shown in the following tabulation indicate that the small-seeded sieva types are susceptible to the lima bean virus, whereas the large-seeded Fordhook sorts are resistant. There seems to be no correlation of pole and bush varieties with susceptibility and resistance.

Variety:	*Reaction* [1]	Variety—Continued	*Reaction* [1]
Burpee Best	−	Hopi	+
Burpee Improved	−	Jackson Wonder	+
Carpenteria	−	King of the Garden	−
Challenger	−	Large White	−
Detroit Mammoth	−	Leviathan	−
Dreer Bush	−	McCrea	−
Dwarf Large White	−	New Wonder	−
Early Jersey	−	Seibert	−
Florida Butter (pole)	+	Sieva	+
Florida Speckled	+	Willow Leaf	+
Fordhook	−	Woods Prolific	+
Henderson Bush	+		

[1] Minus signs (−) indicate resistance; plus signs (+), susceptibility.

TRANSMISSION

SEED

There is no evidence that the lima bean mosaic is carried in the seed. In 1935, seed collected from mosaic plants in the field were planted and allowed to grow until the third trifoliate leaf was fully developed. Of 892 plants from this seed that were carefully examined, none had developed mosaic.

INSECTS

The fact that insects transmit mosaic viruses from one plant to another is so well known that it was not thought necessary to go extensively into this phase of the problem. To demonstrate that the lima bean mosaic is no exception, however, a few experiments were conducted in which two species of aphids, *Aphis gossypii* Glov. and *Myzus persicae* (Sulz.),[6] were employed.

The technique was that usually employed in studies of this kind and briefly was as follows: The aphids were reared in cages, *Aphis gossypii* on cucumbers and *Myzus persicae* on pepper plants. When needed,

[6] These two colonies of aphids were each descended from a single individual and were reared in cages to prevent mixing. The identifications were made by P. W. Mason, of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.

a dozen or more individuals were transferred to mosaic lima bean plants and allowed to feed for about 24 hours. At the end of that time, young lima bean plants were introduced into the cage and the diseased plants cut down with the expectation that the insects would crawl to the healthy plants and feed on them.

The results were so striking as to leave no doubt of the ability of these insects to carry the virus. The symptoms produced were typical of lima bean mosaic, and the damage was even more severe than when the inoculations were made mechanically by rubbing the juice of mosaic-infected plants into the leaves of healthy ones. At the end of 4 days the symptoms appeared, and in 5 days the terminal bud and young trifoliate leaves were badly malformed. Usually some of the plants died a few days later. The experiments with these two species of aphids were repeated several times with identical results. The objective of the experiment having been attained by demonstrating that insects may transmit the disease, no other insects were tried. It is not improbable that the same results might be accomplished with other species of aphids and even with other kinds of insects.

PROPERTIES OF THE VIRUS

The physical properties of the virus studied were (1) aging in vitro, (2) thermal inactivation, and (3) effect of dilution in water. These properties were selected because it was believed that they would be useful in the identification of the virus or in demonstrating its relationship to other viruses to which it is apparently closely related.

AGING IN VITRO

Aging of the expressed juice of mosaic-infected plants may be useful in identifying a virus, if the experiments are carried out under known control conditions. These conditions should duplicate as nearly as possible the ones originally employed in studying the virus with which the virus under investigation is to be compared. Unfortunately no fixed standard has been followed by all investigators. Johnson and Hoggan (*5*) recommended a temperature of 22° C., and that temperature has been employed. The leaves of lima bean plants infected with mosaic were ground to a fine pulp, and the juice was extracted by squeezing it through cheesecloth. The undiluted juice was then stored in a stoppered Erlenmeyer flask in the dark in an incubator held at a constant temperature (22° C.). Preliminary experiments showed that under these conditions the juice was viruliferous for more than 24 hours, and no shorter period of time was tried. At the end of each 24 hours, for a period of 12 days, a small quantity of the juice was removed from the flask, diluted with about 2 parts of water, and used to inoculate 10 small tobacco plants. In order to avoid any possibility of introducing the virus of other plants, as for example, tobacco, a separate dissecting needle wrapped in absorbent cotton was used for each plant. Symptoms appeared in about 5 days on plants inoculated with extracts that had been aged for the first 3 to 4 days. With extracts aged longer than 4 days, the time between inoculation and the appearance of the first symptoms gradually increased with the increase in the length of the aging period. The results of two separate experiments are shown in table 1.

TABLE 1.—*Results of aging lima bean virus extract at 22° C. on infectivity*

Period of aging (days)	Plants inoculated	Plants infected	Period of aging (days)	Plants inoculated	Plants infected	Period of aging (days)	Plants inoculated	Plants infected
	Number	*Number*		*Number*	*Number*		*Number*	*Number*
0	30	30	5	20	20	8	20	1
1	20	20	6	20	13	9	20	0
2	20	20	7	20	12	10	20	0
3	20	20						

THERMAL INACTIVATION

In testing the lima bean virus to determine the temperature producing inactivation, the usual method of subjecting 2 cc of an undiluted plant extract in a glass tube to a constant temperature in a water bath for 10 minutes was employed. At the end of the heating period the extract was rapidly cooled and was then diluted with about 2 volumes of water. Higher dilutions were not employed, since it was shown by McKinney (7) that dilutions of 1 to 100 or more reduced the potency of some virus extracts. The extract was obtained from lima bean plants. The combined results of several experiments are shown in table 2.

TABLE 2.—*Results of heating lima bean mosaic virus extract at different temperatures on infectivity*

Temperature (° C.)	Plants inoculated	Plants infected	Temperature (° C.)	Plants inoculated	Plants infected	Temperature (° C.)	Plants inoculated	Plants infected
	Number	*Number*		*Number*	*Number*		*Number*	*Number*
50	20	10	65	40	25	75	30	0
55	20	13	67.5	30	10	Not heated (controls)	30	30
60	30	22	70	30	0			

EFFECT OF DILUTION

The extract was obtained from the leaves of lima bean plants, and the dilution was made with hydrant water. The combined results of several different experiments are shown in table 3.

TABLE 3.—*Results from diluting lima bean virus extract on infectivity*

Dilution	Plants inoculated	Plants infected	Dilution	Plants inoculated	Plants infected	Dilution	Plants inoculated	Plants infected
	Number	*Number*		*Number*	*Number*		*Number*	*Number*
1:100	30	25	1:2,000	40	17	1:10,000	20	3
1:200	20	16	1:3,000	30	17	1:20,000	20	0
1:500	30	23	1:5,000	30	12	No dilution (controls)	40	35
1:1,000	40	26						

DISCUSSION

The facts presented thus far appear sufficient to warrant the conclusion that there is a destructive and distinctive virus disease that produces characteristic symptoms in certain varieties of lima beans. It has also been suggested that the causal virus is similar to or identical with the cucumber virus of Doolittle (3) (cucumber virus 1 of Johnson).

It would be logical to assume that the lima bean virus might be a member of the group of legume viruses studied by Zaumeyer and Wade (*13*), but in view of the host range shown (table 1) this appears not to be the case.

The results of these investigations show that the lima bean virus produces symptoms similar to those caused by the cucumber and celery viruses on a number of different hosts. The host range of the celery virus has been extensively studied by Wellman (*12*) and was thought by him to be different from that of the cucumber mosaic virus. The celery mosaic virus, however, was later shown by Price (*9*) to be very similar to the cucumber virus and is considered by him to be no more than a strain of the latter.

The lima bean virus is, without much doubt, very similar to the cucumber mosaic virus and the celery virus. It can, therefore, be identified as a member of the cucumber virus group. Because it differs in some respects from the cucumber virus, it remains to be determined whether these differences are sufficient to justify classifying it as a strain of the cucumber virus. It causes a type of primary lesion on the inoculated leaves of tobacco plants (pl. 2, *D*) not found on other leaves of the plants and, so far as the author is aware, such lesions do not occur on leaves of tobacco plants inoculated with the virus from cucumber or celery.

Perhaps the most significant difference between the mosaic of lima bean and that of celery and cucumber is that the lima bean virus will infect *Vicia faba* while the viruses of celery and cucumber do not. No reference has been found to results showing that the celery and cucumber viruses will cause any type of infection on the horsebean. On the other hand, local and systemic infections are obtained when the broadbean is inoculated with the lima bean virus. Although the percentages of local and systemic infections on the broadbean are comparatively low, the symptoms are clear and convincing and back-inoculations on tobacco or lima bean have yielded positive results. These differences seem to support the conclusion that there is a possible strain difference between the cucumber and lima bean viruses.

Wellman (*10*) showed that a temperature of 75° C. for 10 minutes was necessary to inactivate the celery virus. In several experiments conducted by the writer, the lima bean virus was inactivated at 70°, and only a little more than 30 percent of the plants inoculated with extract that had been heated to 67.5° for 10 minutes were infected.

The gradual inactivation of plant viruses on aging in vitro has been regarded by some investigators as significant and useful in their classification. The writer has shown (table 1) that the lima bean virus retained its full potency for 5 days when stored at 22° C. The potency was somewhat reduced in 6 and 7 days and the virus was completely inactivated in 9 days. Only 1 plant out of a total of 20 in two experiments developed symptoms after the extract was aged for 8 days. It was shown by Wellman that the celery virus withstood aging from 6 to 8 days at 18° to 20°, which might be expected because of the lower temperature used. The cucumber virus withstands even less aging, being usually inactivated in 3 to 4 days. The ability of the lima bean virus to withstand longer aging than the cucumber virus would seem to indicate also that it is probably somewhat different from the cucumber virus.

Except for those mentioned, only slight differences can be detected in symptomatology. The cucumber and lima bean viruses have been compared on a number of different hosts and in general they agree closely. The lima bean and cucumber viruses both cause filiformities to the leaves of tomato, while the celery virus does not, according to the work of Wellman (*10*).

Wellman was unable to obtain local lesions or systemic infection of the Black cowpea. In a parallel experiment the writer inoculated the Black cowpea by rubbing the upper surface of the leaf with viruliferous juice of the celery, cucumber, and lima bean viruses, and obtained numerous local lesions in all cases. There appeared to be some slight differences in the characteristics of the lesions but it is not believed that they were sufficiently distinct to signify a difference in symptomatology.

The results of these investigations indicate that the lima bean mosaic is caused by a virus very similar to that which causes cucumber mosaic, but not identical with it. The slight differences observed in symptoms of the suscepts are not believed sufficient to justify designating the causal virus as new and distinct but as a strain of the cucumber virus. The writer would suggest designating it as the lima bean mosaic strain of the cucumber virus.

SUMMARY

A mosaic disease of lima beans, similar to the mosaic of cucumber and celery, is described. The disease differs slightly in symptomatology on several different hosts.

The following varieties of lima beans were found to be susceptible: Jackson Wonder, Hopi, Florida Speckled, Florida Butter, Henderson Bush, Woods Prolific, Willow Leaf, and Sieva. The resistant varieties were Burpee Best, Burpee Improved, Carpenteria, Challenger, Detroit Mammoth, Dwarf Large White, Dreer Bush, Early Jersey, Fordhook, King of the Garden, Leviathan, Large White, McCrea, New Wonder, and Seibert.

The lima bean virus differs slightly from the cucumber virus in symptomatology and in some of its physical properties, such as aging in vitro and inactivation upon heating. However, it does not differ enough from the cucumber virus to be classed as distinct. It is proposed, therefore, that it be designated as the lima bean mosaic strain of the cucumber virus.

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SAND DUNES OF RECENT ORIGIN IN THE SOUTHERN GREAT PLAINS[1]

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INTRODUCTION

In recent years, as a result of cultivation, grazing, and drought, sand dunes have developed on large areas of land throughout the southern Great Plains, especially between the Canadian and Arkansas Rivers. Data secured from an aerial survey also show many such sites scattered through Colorado, Kansas, Nebraska, Wyoming, North Dakota, and South Dakota. Their combined extent is approximately 15,000 acres.

Considerable effort has been expended to control and reclaim sand-dune areas throughout the world—in Palestine, Australia, Germany, France, the United States, and many other countries. The usual treatment is to prevent sand movement through stabilization by means of vegetation (*4*).[3] Often barriers are constructed by artificial means to serve as protection against drifting sand. The relationships between sand dunes and vegetation have been discussed by a number of workers, among whom are Cowles (*2*), Harris (*5*, *pp. 41–46*), and Rempel (*7*).

The physical characteristics of dunes, their formation, shape, size, and movement have been described by Cornish (*1*), Cowles (*2*), King (*6*), Rempel (*7*), and others. Studies of sand movement and field operations to control it have been conducted on coastal, river, and lake dunes where there is a constant supply of sand. So far as can be determined, however, no experimental studies have been made of sand-dune areas in the Great Plains.

Three types of sand dunes are found in the Great Plains. The first and least extensive is the "blow-out" type, which develops around wells or from roads and cattle trails. The second type, with which we are here concerned, develops as a result of the destruction of surface cover. Both of these types as a rule are bare of vegetation and are of recent origin. The third type is formed by wind action on outwash material and is usually fairly well stabilized by native vegetation.

Sand dunes of recent origin are often found near older dunes, which indicates that in times past wind action has spread the outwash considerable distances, though not always in dune form.

Studies dealing with the origin and character of the second type of dune areas and with methods of stabilizing and utilizing them were begun in January 1936 by the Soil Conservation Service near Dalhart,

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[2] Acknowledgment is made to several workers of the Soil Conservation Service in Region 6 for their assistance in making measurements and for their suggestions and interest in the problems. Thanks are given especially to B. H. Hopkins, project manager, Dalhart, Tex., and to J. C. Wilcox, Amarillo, Tex., for their aid and encouragement.

[3] Reference is made by number (italic) to Literature Cited, p. 917.

Dallam County, Tex. Two thousand acres of land were obtained for study, 912 of which were leased. The remainder was obtained through cooperative agreement. The 2,000-acre area fell naturally into three distinct dune plots. After measurements were taken, two plots were found to be strikingly similar (table 1). One of these, plot 1, was used as a work plot, and the other, plot 2, as a check. The third plot was in an earlier stage of dune formation, and the study of dunes on this plot is not reported in this paper.

TABLE 1.—*Slope, land cover, soils, and erosion on dune plots 1 and 2 near Dalhart, Tex., Apr. 23, 1936*

Item	Plot 1	Plot 2	Item	Plot 1	Plot 2
Slope:	*Percent*	*Percent*	Erosion—Continued.		
0 to 2 percent	83	90	Accumulation—Continued.	*Percent*	*Percent*
2 to 8 percent	11	2	Severe, 12 to 36 inches deep	7	8
8 to 12 percent	6	8	Small dunes, 36 to 72 inches high	2	2
Land cover:			Large dunes, over 72 inches high	6	8
Abandoned land	58	54	Removal:		
Pasture; one-third of stand killed	8	1	Moderate, 25 to 75 percent of the A horizon removed	(1)	
Pasture; one-third to two-thirds of stand killed	7	6	Severe, A and upper B horizons removed	22	20
Pasture; over two-thirds of stand killed	27	39	Very severe; lower B horizon, the C horizon, or the parent material, removed	14	6
Soils and subsoils:			Accumulation and removal:		
Soils:			Shallow accumulation, 0 to 6 inches, slight removal	16	6
Amarillo loam	1		Moderate accumulation, slight removal	3	4
Amarillo fine sandy loam	20	32	Shallow accumulation, moderate removal	5	9
Amarillo loamy fine sand	21	19	Moderate accumulation, moderate removal	12	17
Subsoils:			Severe accumulation, moderate removal	5	9
Clay	1	4	Small dunes, moderate removal	5	
Clay (moderately light)	10		Moderate accumulation, very severe removal		7
Sandy clay loam	12	13			
Sandy clay loam (light)	1	4			
Loamy fine sand		9			
Sandy clay loam	14	3			
Dune sand	20	16			
Erosion:					
Accumulation:					
Moderate, 6 to 12 inches deep	(1)	2			
Moderate, 6 to 12 inches deep (hummocky)	(1)	3			

[1] Less than 1 percent.

SAND DUNES ON THE STUDY PLOT

The sand dunes of plot 1 like those of the check plot are on land that had not been plowed before the formation of the dunes. They developed as a result of wind action on an 80-acre field that lies to the southwest of the plots. This field was cultivated from 1907 to 1914 and was then used for grazing. Dry years and heavy grazing of the field prevented growth of sufficient vegetation to hold the soil. According to the best information obtainable,[4] it was in 1926 that small hummocks of sand were first noticed to be forming against fence rows and piling against buildings, and unpalatable plants (Russian-thistle and soapweed) began to appear.

After 1929 striking growth of the dunes was noticed. Instead of forming on the original broken sod, however, the dunes formed to the north and east, since the prevailing winds are from the southwest. As years passed the large dunes shifted farther north and east, leaving large, completely denuded areas behind (fig. 1). The coalescence of

[4] From W. H. Lathem, Land Office, Dalhart, Tex.

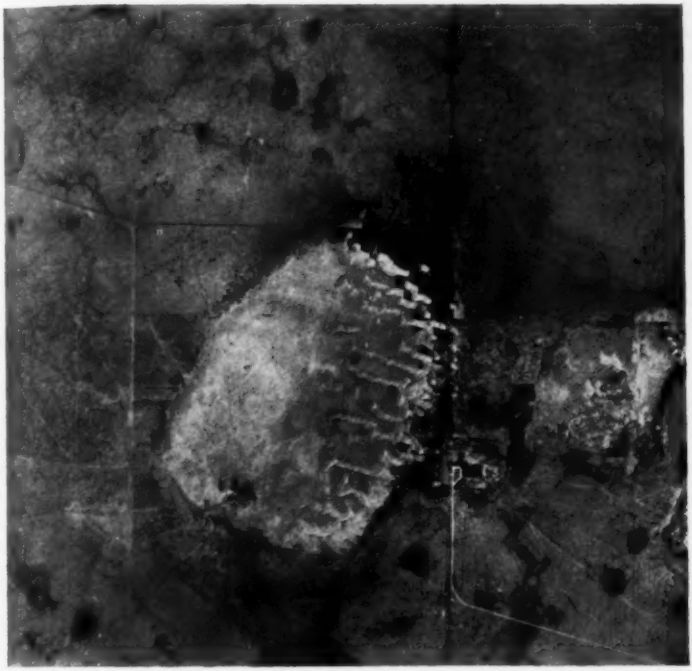

FIGURE 1.—An airplane view of a typical dune site, October 1936. The sand dunes are east and north of the denuded area.

dunes caused a great increase in their height as well as in their length and width.

Usually the dunes have long, gently rising windward slopes and crescent-shaped leeward slopes (fig. 2). Dunes as low as 6 feet may

FIGURE 2.—A typical sand dune in the background. The windward slope is long and gently rising; the leeward slope is much more abrupt and generally crescent-shaped.

take this form and may continue to build up to greater heights when weeds become lodged on them or the packing of the sand prevents it from blowing. There is some evidence that crescentic sand dunes form most readily on hard, level substratum. There is also evidence

that the crescent shape of the leeward side accounts for the increase in size of the dune. When the wind approaches the top of the dune it drops its load of sand as a result of the formation of eddies or wind currents that move at right angles to the prevailing winds.

ORIGINAL AND PRESENT VEGETATION

Originally, the entire study plot was probably dominated by a mixed type of vegetation. Blue grama (*Bouteloua gracilis* (H. B. K.) Lag.) and buffalo grass (*Buchloë dactyloides* (Nutt.) Engelm.) were undoubtedly the conspicuous species on the gently rolling slopes, and side-oats grama (*Bouteloua curtipendula* (Michx.) Torr.) and silver beardgrass (*Andropogon saccharoides* Sw.) were found in depressions. Red three-awn (*Aristida longiseta* Steud.) and sand dropseed (*Sporobolus cryptandrus* (Torr.) A. Gray) were species on immature soils and in disturbed places. If plants of sand sage (*Artemisia filifolia* Torr.) and *Yucca glauca* Nutt. were present, they were probably few.

The conspicuous grass at present is blue grama, but relict plants of buffalo grass and the mid and tall grasses occur over the area.

THE SAND-DUNE COMPLEX ON PLOTS 1 AND 2

It must be kept in mind that plots 1 and 2 were similar in practically every respect to begin with, and that plot 1 is the plot on which the dunes were stabilized and plot 2 is the check plot. On both of the plots a typical sand-dune complex consists of four distinct divisions: (1) The sand dunes; (2) eroded land, which occurs to the west of and between the dunes; (3) surrounding vegetated land; and (4) a border area that separates the sand dunes and eroded land from the adjacent less affected portion.

THE SAND DUNES

The sand dunes range from 50 to 770 yards in length and are generally 30 to 50 yards wide (fig. 3). Their height depends, appar-

FIGURE 3.—Sand dunes alternate with areas of hard, blown-out land. This dune is approximately 22 feet in height.

ently, on their age and the direction and velocity of the wind. Data regarding dunes on plot 1 in 1935–36 are given in tables 2 and 3. The height of the highest dune measured in 1936 was 26 feet (table 3). The axis of most of the dunes is east and west, but in some it may be a little southeast and northwest, owing to the direction of the prevailing wind. The dunes are irregularly spaced, the distance between them ranging from 50 to 400 yards. They are generally compact beneath and moist a few inches below the surface.

TABLE 2.—*Number of dunes, classified as to length and width, on plot 1, 1935–36*

Dimensions and year	0–100 feet	101–300 feet	301–500 feet	501–700 feet	701–900 feet	901–1,100 feet	1,101–1,600 feet	1,601–2,400 feet	Total dunes
Length:	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
1935	3	19	14	6	7	5	0	0	54
1936	0	5	1	3	2	0	2	1	14
Width:									
1935	17	37	0	0	0	0	0	0	54
1936	2	6	6	0	0	0	0	0	14

TABLE 3.—*Height of 6 dunes on plot 1, 1935–36* [1]

Year	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
	Feet	*Feet*	*Feet*	*Feet*	*Feet*	*Feet*
1935	12	12	14	14	15	18
1936	21	24	18	25	26	21

[1] Height of all dunes ranged from 1 to 19 feet in 1935, and from 2 to 26 feet in 1936.

THE SUBSTRATUM

The substratum on which the dunes rest, as well as that between and around them, is very compact, hard, and fairly level. It consists of the subsoils of uncultivated land that has been eroded to various depths, in some places to as much as 4 feet. The fact that the prevailing winds are from the southwest accounts for there being large areas of hard substratum to the west and south of the dunes and accumulations of sand from 6 inches to 2 feet in depth to the north and east. In places this hard land assumes the appearance of desert pavement, owing to the exposure of caliche and other fragments too heavy to be moved by the wind.

THE CRITICAL AREA

The critical area is an ecotone or border strip that lies between the range grassland and the eroded substratum and dune lands. This border is designated "critical area" because of its hummocked condition and the presence of piles of loose sand devoid of vegetation that make it a possible source of materials for dune development, and because it possesses other characteristics that apparently aid in dune formation, such as vulnerability to the winds that sweep unhindered across the hard land, picking up material as they go.

THE MOVEMENT OF DUNES

Small dunes or mounds coalesce to form large dunes. In June 1935, according to a survey of the Operations Division of the Soil Conservation Service project at Dalhart, Tex., there were on plot 1

some 50 small dunes from 2 to 18 feet in height and of various lengths and widths. Although some were merging, most of them were distinct. In June 1936 on the same plot there were only five relatively large dunes and nine outlying smaller ones (table 2).

When dunes are small, their rate of movement is very rapid. Measurements over a 2-week period showed that one dune moved as much as 26 feet. Most of this movement undoubtedly occurred in the course of the one storm lasting several hours that took place during this period.

When dunes reach a height of more than 16 feet, their movement is less rapid. Measurements of the larger dunes, however, showed that they had progressed in a northeast direction an average of 37 feet in 1 year, the sand being rapidly removed from the west end of the dunes and added to other dunes to the north and east.

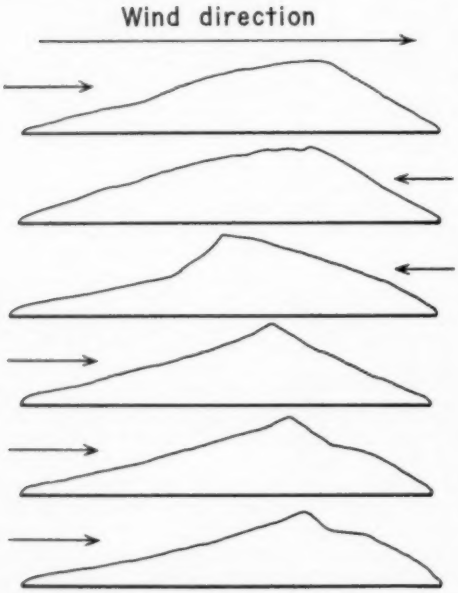

FIGURE 4.—Effect of reversal of wind on cross section of a dune. (After King (6).)

The rate of wind movement is one of the most influential factors in the moving of sand, but this force is definitely modified by local conditions. The greatest shifting of materials occurs during the spring blowing season and when wind velocities are above 20 miles per hour. As a rule, the higher the velocity of the wind the greater the movement of the sand; but when the land is in condition to blow, as it often is in February, March, and April, winds of low intensities move large quantities of material.

Since the prevailing direction of the wind is from the southwest, the dunes are moving in a northeast direction. Severe windstorms, however, come out of the north and west, and although they fail to change the location of the larger dunes, the slopes may be completely reversed, the steep leeward slope facing southwest and the windward slope facing northeast (fig. 4).

STABILIZATION OF DUNES

Since large machinery is expensive and is not available to all farmers and ranchers, it was necessary to develop economical as well as efficient methods by which the sand-dune areas could be stabilized. One practical solution lay in the utilization of wind power to redistribute the materials that this same force had built up into dunes. This principle has been employed in practically all the work.

METHODS OF DECREASING HEIGHT OF DUNES

In order to decrease the height of dunes sufficiently to permit their being effectively planted, which is necessary for stabilization, four means of facilitating movement were employed, namely, (1) wind intensifiers, (2) drag-pole, (3) one-way disk, and (4) tractor and blade.

Wind Intensifiers

Three types of intensifiers were tried, the signboard (fig. 5), the gunny sack, and the wind channel. The signboard type was constructed by nailing galvanized iron or boards between posts at different heights above the sand level on the crest of the dune. Gunny sacks were filled with sand and put at different spacings on top of the dune. Wind channels were dug in the dune; these were generally 3 feet wide and 4 feet deep.

The wind intensifiers were placed at the crest of the dune. They proved very efficient in moving sand; large gaps 4, 5, and 6 feet deep

FIGURE 5.—Signboard type of intensifier made of boards and galvanized iron and placed on the crest of the dune.

were dug out and the sand was carried out beyond the crest. The sandbags were most effective, since they would lower as the wind removed the sand from around them. During one storm, a dune was lowered about 2 feet by the use of sandbags. The crest of the dune flattened down, and the dune itself moved forward 6 to 10 feet. The use of wind intensifiers in lowering dunes did not prove to be practical, however, because too much manual labor was required both in setting them up and in digging out those that had been undermined and had toppled over or were totally or partly covered by the moving sand during heavy windstorms.

Drag Pole

A second means of destroying the steep slope to the leeward side of the dunes, and thus allowing the wind to carry the sand out beyond, is the use of a drag pole. The drag pole is an 8- by 8-inch timber of

sufficient length (for the largest dunes, 20 feet). One or two horses are hitched to each end of the pole and it is dragged along the sharp edge of the dune at right angles to the crest. Attaching a disk at the top aids in breaking down the steep slope.

The drag pole can be used more advantageously than the wind intensifiers because it requires less hand labor and a greater area can be covered in a shorter period of time. By this means, as by the use of the intensifiers, the wind is prevented from forming eddies and as a result it carries huge quantities of sand out beyond the dune. The dune seems to move en masse when the crest is broken down by this method. One dune on which the drag pole was used was lowered 15 feet in 6 months.

Of the four methods here suggested for decreasing the height of dunes, the use of the drag pole is the most efficient as well as the most economical. Furthermore, as this method does not require the use of expensive machinery, it is practicable for the farmer and rancher.

One-Way Disk

The sandy material making up a dune often becomes compacted, owing to vegetative growth, trampling of stock, and rain. The best means yet found to loosen this sand so that it can be moved by wind action is a one-way disk plow. Disk-harrowing the dunes with teams is economical as well as effective.

Tractor and Blade

Another method employed to spread the sand is the use of a tractor and blade. By this means dunes can be flattened and leveled, and when it is supplemented by wind action, quick results are obtained. One dune worked in this way was lowered from 20 feet to 5 feet in 6 months and at the end of that time was in condition to be planted (fig. 6).

The procedure with the blade is to make one to three turns over the highest points of the sand dune, after which the equipment is moved to another dune which is treated similarly. This treatment gives the wind a chance to move the loose sand. From 6 to 12 of these treatments are generally necessary to affect materially the height of the dune.

LISTING

One method employed to prevent the sand's moving from one dune to accumulate on another is that of listing the hard land around and between the dunes (fig. 6, *A*). This procedure prevents more sand from accumulating on the dune and also catches the material blown off the dune. The need for deep listing cannot be overemphasized. Shallow-listed areas have continued to blow, whereas the deep-listed lands on the experimental areas have not blown. Relisting has been done over much of the area, and the soil, even that which was badly eroded, has been mixed with sand and other wind-blown material to such an extent that it is probable that a crop can be produced on most of it.

STABILIZATION THROUGH VEGETATION

The development of a vegetative cover, whether cultivated crop or native vegetation, is necessary for complete stabilization.

The critical area between the hard eroded land of the study plot and the native pasture was a mass of loose, drifting sand, which was often hummocked by the Russian-thistle that grew there. Listing this area and planting it to grain sorghums held the sand and prevented it from

FIGURE 6.—*A*, An isolated dune with the typical half-moon shape. The hard land around this dune has been listed. *B*, The man is standing approximately in the place occupied by the car in *A*. This dune has practically coalesced with the one to the north. It has been lowered approximately 15 feet.

being blown across the hard land to cause further erosion and accumulation.

As a result of listing the hard eroded lands and cutting off some of the mechanical action of the wind by treating the critical area, vegetation developed on many of the areas that were formerly bare. Although this cover was mainly Russian-thistle, it was sufficient to

prevent blowing and even to catch and hold the moving sand. On the check plot no weeds appeared on the bare land.

The fine sandy soils composing the dunes, when properly cultivated and supplied with sufficient moisture, are capable of producing the abundant vegetation that is essential to permanent stabilization. After the dunes had been lowered by mechanical means to a height that permitted their being effectively planted, various row-crop species, Sudan grass, milo, kafir, and hegari, were drilled and listed over and around the dunes, and over most of the area a sufficient stand was produced to protect the soil adequately (fig. 7).

FIGURE 7.—Site in figure 5, about 14 months later. The original view was taken from the ground; this picture was taken from the top of an automobile. Good stands of Sudan grass, black amber cane, and kafir have grown on this once badly eroded area. Both the hard substratum and the sand dunes now have a cover adequate to protect the soil from further erosion during the coming year.

Hay of sand reedgrass (*Calamovilfa gigantea* (Nutt.) Scribn. and Merr.) was spread over one dune, 1.1 acres in extent and more than 16 feet high, in June 1936. By July 1937 a dense growth of sand reedgrass and Russian-thistle completely covered this area, the Russian-thistle dominating for the most part on the drier southwest exposure. The grass plants were of good height and formed a relatively thick stand.

SUMMARY AND CONCLUSION

Erosion hazards in the form of sand dunes have developed in recent years on large areas of land throughout the Great Plains. In January 1936, the Soil Conservation Service initiated studies to determine the best methods of stabilizing and utilizing such sites. To accomplish this objective it was necessary to investigate the origin and character of sand dune areas.

As a result of these studies it was found that the sand dunes could be effectively lowered by utilizing the force of the wind to redistribute the materials that this same force had built up into dunes and that

over most of the area a sufficient cover crop could be produced to protect the soil adequately against further erosion.

This study indicates that the better land is capable of producing good crops of grain sorghums if farmed in such a way as to prevent soil drifts (*3*) and also that these dune sites can be returned to grass.

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THE EFFECT OF THE X FACTOR, OF SODIUM CHLORIDE, AND OF THE COMPOSITION OF THE NUTRIENT MEDIA UPON THE GROWTH OF THE FOWL CORYZA BACILLUS, HEMOPHILUS GALLINARUM [1]

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INTRODUCTION

The recent work of Kessens [2] concerning the nature of the growth requirements of the European fowl coryza bacillus indicates the need of the V factor and an additional factor, which he terms the C, or coryza factor, for its growth. Kessens found that the clotted-blood serum of the chicken and pigeon contains X, V, and C factors, and thus meets the requirements for growth, while that of the horse, ox, sheep, goat, rabbit, and cat possess only the X factor and will not support growth. The C factor of chicken serum was destroyed by heating at 90° to 100° C. for 10 minutes. Chicken serum so heated supported the growth of *Haemophilus influenzae*, but it was unsuitable for the coryza bacillus (H. *gallinarum*), although it still possessed the X and V factors. As a result of these studies, Kessens suggests the name *Haemophilus coryzae* for the organism inasmuch as the X factor was not required for growth.

Schalm and Beach [3] report the need of the X and V factors for growth of the American strains of the fowl coryza bacillus which they studied.

The writers [4] observed a considerable difference in the amount of growth that occurred with the coryza bacillus when chicken blood was used at the base of nutrient agar slants and when it was used in the form of a blood-broth medium. The writers [5] also obtained growth with serum from clotted horse blood diluted in the proportion of 1 : 5 with broth at the base of agar slants but not with the same diluted serum broth when used alone.

The experiments herein described were conducted to determine what effects the use of the test-fluid medium at the base of agar slants, as compared to its use alone, would have on the growth requirements of the Rhode Island strains of the fowl coryza bacillus as a result of the difference in growth cited.

[1] Received for publication September 22, 1937, issued June 1938. Contribution No. 510 of the Rhode Island Agricultural Experiment Station.

[2] KESSENS, B. H. VERGELIJKEND ONDERZOEK BETREFFENDE HAEMOPHILUS CORYZAE, HAEMOPHILUS INFLUENZAE EN ANDERE HAEMOPHIELE BACILLEN. Rijks Univ., Utrecht. 1936.

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[4] DELAPLANE, J. P., ERWIN, L. E., and STUART, H. O. A HEMOPHILIC BACILLUS AS THE CAUSE OF AN INFECTIOUS RHINITIS (CORYZA) OF FOWLS. R. I. Agr. Expt. Sta. Bull. 244, 12 pp., illus. 1934.

[5] Unpublished data.

EXPERIMENTAL DATA

GROWTH FACTORS IN YEAST

Since Thjötta and Avery [6] found that yeast supplies the V factor for *Hemophilus influenzae* yeast extracts and yeast-cell suspensions were prepared to determine their value in supplying the factor necessary for growth of the fowl coryza bacillus. If the organism grew in the yeast media, inoculations for the various tests would be made from them and thus the possibility would be avoided of a carry-over of the growth factors from the blood cultures that otherwise would have to serve as the source of inoculum.

Boiled acidulated yeast extract supplies the V factor. Since Kessens [7] found that the C factor of chicken serum was destroyed by heating for 10 minutes at 90° to 100° C., the question arose as to whether this factor might not have been destroyed in preparing the boiled yeast extract.

Pure dry Fleischmann's irradiated yeast was prepared as follows: 20 g of yeast was added to 100 cc of distilled water and stirred until as much as possible of the yeast was in suspension. The material was then centrifuged and a yellowish, clear, transparent, supernatant extract was obtained. A few drops of phenol red were added to the extract, and sodium hydroxide was used to adjust the pH until it was slightly alkaline to this indicator. The extract was again centrifuged and passed through Seitz filters. The sterile yeast extract was added to the base of nutrient agar slants (Difco dehydrated nutrient agar, 23 g; sodium chloride, 8 g; water, 1,000 cc).

Acidulated yeast was prepared according to the method of Thjötta and Avery,[8] except that the filtrate, after boiling, was made slightly alkaline to phenol red by the addition of sodium hydroxide, then centrifuged, and passed through Seitz filters.

Autoclaved blood extract was prepared according to the method described by Schalm and Beach.[9] The blood of chickens, sheep, goats, and horses was used. Autoclaved blood extract contains only the X factor.

Pure cultures of yeast were used to determine whether suspensions of the yeast might supply some of the growth requirements. Yeast was grown on beerwort agar, and the cells were washed into suspension with sterile physiological saline solution. The pH of the suspension was adjusted to the neutral point as indicated by phenol red.

Two Rhode Island strains of the organism were used for these studies, cultures 44 and 80. Culture 44 had been cultured for over 3 years in the writers' laboratory; culture 80 had only recently been isolated from a field type of coryza.

Growth occurred in the first tubes of yeast extract (unheated) when inoculated from the stock cultures (table 1). Attempts to continue the culture in this type of extract by inoculating from the successful ones, failed after several attempts. However, upon the addition of a loopful of chicken red blood cells to each tube, growth occurred after inoculation, as in the first tubes inoculated from the stock cultures.

[6] THJÖTTA, T., and AVERY, O. T. STUDIES ON BACTERIAL NUTRITION. II. GROWTH ACCESSORY SUBSTANCES IN THE CULTIVATION OF HEMOPHILIC BACILLI. Jour. Expt. Med. 34: 97–114. 1921.
[7] KESSENS, B. H. See footnote 2.
[8] THJÖTTA, T., and AVERY, O. T. See footnote 6.
[9] SCHALM, O. W., and BEACH, J. R. See footnote 3.

The addition of autoclaved blood extract from the chicken, sheep, goat, and horse to the yeast extract also completed the growth requirements (table 1).

The boiled yeast extract plus autoclaved blood extract gave only a poor growth, indicating the destruction of some substance as a result of boiling. Since Kessens [10] called this the C factor, it will be so refered to here.

TABLE 1.—*Growth of the fowl coryza bacillus in yeast extract and in yeast suspension at the base of nutrient agar slants*

Inoculum	Medium	24- and 48-hour microscopic examination [1]		Growth in check medium [2]	
		Culture 44	Culture 80	Culture 44	Culture 80
Cultures 44 and 80 grown in chicken blood.	Yeast extract (unheated)	++	++	++	++
	do	−	−	−	−
First yeast-extract culture.	Yeast extract (unheated) and chicken red blood cells.	++	++	++	++
Yeast extract culture	Yeast extract and autoclaved chicken blood extract.	++	++	++	++
	Yeast extract and autoclaved sheep-blood extract.	++	++	++	++
	Yeast extract and autoclaved goat-blood extract.	++	++	++	++
	Yeast extract and autoclaved horse-blood extract.	++	++	++	++
	Boiled yeast extract and autoclaved chicken blood extract.	±	±	++	++
	Yeast-cell suspension	−	−	−	−
	Yeast-cell suspension plus autoclaved blood extract.	±	±	±	±
	Autoclaved blood extract [3]	−	−	−	−

[1] +=growth; ++=good growth; ±=poor growth; −=no growth.
[2] Check medium consisted of chicken blood at the base of nutrient agar slants.
[3] Chicken, sheep, goat, and horse red blood cells used.

It is evident that yeast extract (unheated) possesses both the V and the C factors, which alone are insufficient for growth. It meets the requirements for growth upon the addition of blood or autoclaved blood extract containing the X factor.

Boiled yeast extract contains the V factor and very little of the C factor, as indicated by the results obtained with this preparation plus autoclaved blood extract. Thus, the presence of the X and V factors do not suffice for good growth of the fowl coryza bacillus. Growth is good, however, in the presence of the X, V, and C factors, as indicated by the use of the unheated yeast extract (line 1, table 1).

The growth obtained from the inoculation of the yeast extract from the stock blood culture can probably be explained on the assumption that sufficient of the X factor was carried over in the inoculum. Thjötta and Avery [11] noted this in their studies of *Hemophilus influenzae*.

The autoclaved blood extract failed to give any growth, showing that other factors, in addition to the X factor are essential for growth (table 1).

Suspensions of the yeast cells plus autoclaved blood extract met the growth requirements. The suspension of yeast cells failed in this respect (table 1).

[10] KESSENS, B. H. See footnote 2.
[11] THJÖTTA, T., and AVERY, O. T. See footnote 6.

GROWTH FACTORS IN CLOTTED-BLOOD SERUM OF HORSE, SHEEP, GOAT, COW, RABBIT, PIG, TURKEY, AND CHICKEN

In each instance the serum employed in these studies was obtained from only one animal of a species, except that of the chicken. The blood was collected in dry, sterile glass bottles and permitted to clot without any agitation. The serum was removed, centrifuged, and passed through Seitz filters for sterilization.

The serum for each test was added to the broth [12] in the proportion of 1:20.

Some of each serum-broth mixture was placed in sterile tubes and some at the base of nutrient agar slants. All were inoculated from cultures maintained in yeast extract plus autoclaved blood extract at the base of nutrient agar slants.

Slides for microscopic examination were prepared from each culture after 24 and 48 hours' incubation at 37° C. After 48 hours' incubation, material from each tube was used to inoculate sterile citrated chicken blood at the base of agar slants to check for growth and for any contamination that might arise.

Growth did not occur in the serum broth tubes of any of the animal serums except those of the chicken and turkey (table 2). The serum broth of each animal at the base of nutrient agar slants showed growth in each instance, as indicated by the fact that the organisms were recovered in citrated chicken blood at the base of nutrient agar slants. Growth was particularly good in the serum of the chicken, turkey, sheep, and goat, and was poor in that of the horse, cow, rabbit, and pig. In the case of the cow and pig serums, growth was evident only at the time of the examination of the chicken blood which had been inoculated from these particular cultures. These results confirm observations previously made by the writers [13] that with chicken blood there is an appreciable difference in the amount of growth obtained in blood broth alone and that obtained in the same material at the base of nutrient agar slants.

The question might arise as to whether the organisms grew or whether they merely survived in certain of the serums inasmuch as recovery of the organisms in chicken blood was used as an indication of growth. In no instance was growth obtained from the serum broth tubes or from the control broths; so it must be concluded either that growth did occur, or that the material at the base of the agar slants was more suitable for the maintenance of the organisms.

In the case of the sheep, goat, chicken, and turkey serums the broth in dilutions of 1:20 at the base of nutrient agar slants was sufficient for continued growth when transfers from the respective tubes were used to inoculate similar ones. Such was not the case with the serums of horse, cow, rabbit, or pig, as growth could not be continued. These tests indicate that the serum of the particular animal employed varied in the concentration of the growth factors when it was used at the base of nutrient agar slants and, except in the case of the chicken and turkey, that it would not support growth when used alone.

Serum from clotted chicken blood diluted 1:20 with broth was boiled 10 minutes, permitted to cool at room temperature, and transferred to sterile tubes and to the base of nutrient agar slants. There was no growth in the heated serum broth in sterile tubes, but in the

[12] Broth, except where the sodium chloride varied in different tests, was prepared as follows: Beef extract, 5 g; peptone, 5 g; salt, 5 g; and water, 1,000 cc.

[13] DELAPLANE, J. P., ERWIN, L. E., and STUART, H. O. See footnote 4.

same material at the base of nutrient agar slants, growth occurred (table 2). Apparently some change took place as a result of boiling, but the fact that growth occurred at the base of the agar slants indicates that the destruction was not complete.

TABLE 2.—*Growth of the fowl coryza bacillus when serum from the clotted blood of various animals was used in broth at the base of nutrient agar slants and when used in broth alone.*

Medium	24- and 48-hour microscopic examination [1]		Growth in check medium [1 2]	
	Culture 44	Culture 80	Culture 44	Culture 80
Horse serum and broth (1:20)	−	−	−	−
Horse serum and broth (1:20) at base of agar slants	±	±	++	++
Cow serum and broth (1:20)	−	−	−	−
Cow serum and broth (1:20) at base of agar slants	−	−	++	++
Sheep serum and broth (1:20)	−	−	−	−
Sheep serum and broth (1:20) at base of agar slants	+	+	++	++
Goat serum and broth (1:20)	−	−	−	−
Goat serum and broth (1:20) at base of agar slants	++	++	++	++
Rabbit serum and broth (1:20)	−	−	−	−
Rabbit serum and broth (1:20) at base of agar slants	±	±	++	++
Pig serum and broth (1:20)	−	−	−	−
Pig serum and broth (1:20) at base of agar slants	−	−	++	++
Turkey serum and broth (1:20)	±	±	++	++
Turkey serum and broth (1:20) at base of agar slants	++	++	++	++
Chicken serum and broth (1:20)	+	+	++	++
Chicken serum and broth (1:20) at base of agar slants	++	++	++	++
Chicken serum and broth (1:20) boiled 10 minutes	−	−	−	−
Chicken serum and broth (1:20) boiled 10 minutes, at base of agar slants	+	+	++	++
Control broth	−	−	−	−
Control broth at base of agar slants	−	−	−	−
Yeast extract and autoclaved red-blood cells	−	−	−	−
Yeast extract and autoclaved red-blood cells at base of agar slants	++	++	++	++

[1] +=growth; ++=good growth; ±=poor growth; −=no growth.
[2] Check medium consisted of chicken blood at the base of nutrient agar slants.

After noting the difference in reaction of the various serums when used with broth alone and when used with broth at the base of agar slants, the writers made another test in which yeast extract plus autoclaved blood extract was used in sterile tubes and at the base of nutrient agar slants. No growth occurred in the sterile tubes (table 2) but at the base of the agar slants growth was good.

THE POSSIBLE ROLE OF AGAR AS USED IN THESE EXPERIMENTS

Since it was apparent that there is a difference between growth of the Rhode Island fowl coryza bacillus in serum broth and in serum broth at the base of agar slants, experiments to determine the nature of these differences were conducted. Since it had also been noted [14] that differences in growth could be detected with different percentages of sodium chloride in the agar when citrated chicken blood was used at the base of the agar slants. Accordingly an experiment was made in which a medium containing 1.5 percent of plain agar (without the addition of nutrients) was compared with a similar medium to which 0.8 percent of sodium chloride was added. The serum of sheep and goat diluted 1:20 with broth was used at the base of each type of agar slant. Yeast extract plus autoclaved red-blood extract was also used. No growth occurred at the base of the nonnutrient agar slants in either the serum or the yeast medium, but growth did occur at the base of the nonnutrient salt agar slants (table 3).

[14] Unpublished data.

TABLE 3.—*Growth of the fowl coryza bacillus when 1.5 percent of nonnutrient agar and 1.5 percent of nonnutrient agar plus 0.8 percent of sodium chloride were substituted for nutrient agar*

Medium	24- and 48-hour microscopic examination [1]		Growth in check medium [2]	
	Culture 44	Culture 80	Culture 44	Culture 80
Yeast extract and autoclaved blood extract at base of nonnutrient agar slants	−	−	−	−
Yeast extract and autoclaved blood extract at base of nonnutrient salt agar slants	+	+	++	++
Sheep serum and broth (1:20) at base of nonnutrient agar slants	−	−	−	−
Goat serum and broth (1:20) at base of nonnutrient agar slants	−	−	−	−
Sheep serum and broth (1:20) at base of nonnutrient salt agar slants	+.	+	++	++
Goat serum and broth (1:20) at base of nonnutrient salt agar slants	++	++	++	++

[1] +=growth; ++=good growth; −=no growth.
[2] Check medium consisted of chicken blood at base of nutrient agar slants.

TABLE 4.—*Effect on growth of the fowl coryza bacillus of the addition of 2 percent of sodium chloride to the growth medium*

Medium	24- and 48-hour microscopic examination [1]		Growth in check medium [2]	
	Culture 44	Culture 80	Culture 44	Culture 80
Horse serum and 2 percent NaCl broth (1:20)	−	−	−	−
Cow serum and 2 percent NaCl broth (1:20)	−	−	−	−
Sheep serum and 2 percent NaCl broth (1:20)	+	+	++	++
Rabbit serum and 2 percent NaCl broth (1:20)	−	−	−	−
Goat serum and 2 percent NaCl broth (1:20)	+	+	++	++
Pig serum and 2 percent NaCl broth (1:20)	−	−	−	−
Turkey serum and 2 percent NaCl broth (1:20)	+	+	++	++
Chicken serum and 2 percent NaCl broth (1:20)	++	++	++	++
Horse serum and 2 percent NaCl broth (1:5)	+	+	++	++
Cow serum and 2 percent NaCl broth (1:5)	−	−	+	+
Rabbit serum and 2 percent NaCl broth (1:5)	−	−	++	++
Pig serum and 2 percent NaCl broth (1:5)	−	−	−	−
Yeast extract, and 2 percent NaCl, and autoclaved red blood cells	−	−	−	−
Yeast extract, 2 percent NaCl, and autoclaved red blood cells at base of nutrient agar slants	++	++	++	++

[1] +=growth; ++=good growth; −=no growth.
[2] Check medium consisted of chicken blood at base of nutrient agar slants.

Table 4 shows the results of studies in which a 2-percent sodium chloride broth was used with the serum of the various animals in the proportion of 1:20 to determine whether the agar could be completely replaced by it. The 1:20 dilutions of the horse, cow, rabbit, and pig serums did not support growth, but the goat, sheep, turkey, and chicken serums did support it. Later, the serum from the horse, cow, rabbit, and pig were used with a 2-percent sodium chloride broth in the proportion of 1:5. No growth could be detected microscopically except in the horse serum, but the organisms were recovered from the cow and rabbit upon the inoculation of chicken blood at the base of nutrient agar slants. Yeast extract plus 2 percent sodium chloride and autoclaved blood extract failed to show growth, but when the same material was used at the base of nutrient agar slants growth occurred.

To determine the optimum proportion of sodium chloride for growth of the organism, chicken serum in the proportion of 1:20 in broth without salt, and in broth containing 0.5, 0.8, 1.5, 2.0, 2.5, 3.0 and 3.5 percent of salt was tested. Chicken serum diluted with broth in the proportion of 1:20 without salt was used at the base of nutrient agar slants to which no sodium chloride was added and with the nutrient agar containing the same percentage of salt as the broths mentioned above.

TABLE 5.—*Growth of the fowl coryza bacillus in chicken serum broth (1:20 dilution) and on nutrient agar when different percentages of salt were added*

Chicken serum [1] diluted (1:20) in broth plus—	Microscopical examination after [2]—			
	24 hours		48 hours	
	Culture 44	Culture 80	Culture 44	Culture 80
No NaCl	−	−	−	−
0.5 percent NaCl	+	+	+	+
Nutrient agar, no NaCl	−	−	−	−
Nutrient agar plus 0.5 percent NaCl	+	+	+	+
Broth plus 0.8 percent NaCl	+	+	+	+
Nutrient agar plus 0.8 percent NaCl	+	+	+	+
Broth plus 1.5 percent NaCl	+	+	++	++
Nutrient agar plus 1.5 percent NaCl	+	+	++	++
Broth plus 2 percent NaCl	++	++	++	++
Nutrient agar plus 2 percent NaCl	++	++	++	++
Broth plus 2.5 percent NaCl	±	±	±	±
Nutrient agar plus 2.5 percent NaCl	±	±	±	±
Broth plus 3 percent NaCl	×	×	×	×
Nutrient agar plus 3 percent NaCl	±	±	±	±
Broth plus 3.5 percent NaCl	×	×	×	×
Nutrient agar plus 3.5 percent NaCl	×	×	×	×
Control broths, no serums [3]	−	−	−	−

[1] Chicken serum diluted 1:20 with broth without salt was used at the base of the nutrient agar slants.
[2] +=growth; ++=good growth; −=no growth; ±=poor growth; ×=growth obtained in chicken blood at the base of nutrient agar slants after being inoculated from these test media.
[3] Controls consisted of broth with and without the respective amounts of salt used in each instance.

The results (table 5) indicate that salt is essential for growth. The growth ranges from the 0.5 percent salt in the broth and agar up to 2.5 percent for the broth and to 3.0 percent for the agar, the optimum concentration ranging between 1.5 and 2.0 percent for both the broth and agar.

These tests indicate that sodium chloride contributes to the growth of the organisms as employed, but does not completely take the place of agar. That nutrients of the nutrient agar are not necessarily essential is also evident from these trials. Whether other salts could replace the sodium chloride has not been determined.

SUMMARY AND CONCLUSIONS

The need of the X factor for the growth of the Rhode Island strains of the fowl coryza bacillus is indicated by the failure of the organism to grow in yeast extract or yeast suspensions at the base of nutrient agar slants unless this factor is supplied in some manner, as by the addition of autoclaved blood extract, or when the medium used to inoculate the yeast extract is of blood and sufficient of the X factor is carried over in the inoculum.

Growth on yeast extract prepared by boiling was poorer than that on yeast extract prepared without heat. Yeast-cell suspensions plus autoclaved blood extract supported growth. Yeast-cell suspensions alone did not.

The clotted-blood serum of the horse, cow, sheep, goat, pig, and rabbit failed to support growth when employed in dilutions of 1:20 in broth, but the same serum broth supported growth when used at the base of nutrient agar slants. This indicates that the growth factors were not so concentrated in the serum of these animals as in those of the chicken and turkey, but that this was partly compensated for when nutrient agar was employed; although, as shown by the controls, neither the agar nor the broth was capable of supplying these requirements. The serum of the horse, cow, rabbit, and pig in a dilution of 1:20 at the base of agar slants failed to give continued growth when transfers were made to additional tubes of the same serum medium, whereas continued cultivation was possible in the case of the sheep, goat, turkey, and chicken serums.

A study of the role of agar in growth indicated that sodium chloride in the proportions used could partly but not completely replace agar in furnishing the requirements necessary for growth.

That the factors in chicken serum in a dilution of 1:20 are adversely affected by boiling but are not completely destroyed is indicated by the fact that some growth occurred when the serum was used at the base of nutrient agar slants.

The results of the tests with the serum of horse, sheep, goat, cow, and rabbit, when used in the dilution of 1:20 in broth, were similar to those reported by Kessens.[15] That the same serum broths at the base of agar slants supported growth in some degree indicates that they were not entirely free from the growth factors.

The results of the use of unheated yeast extract indicate that *Hemophilus gallinarum* will not grow when only the V and C factors are present. The need of the X factor is indicated by the results obtained when autoclaved blood extract was added to the yeast extract. Boiled yeast extract plus autoclaved blood extract does not support good growth although it contains the X and V factors.

Considerable differences in growth were noted in yeast extracts and serum broths when used alone and when used at the base of nutrient agar slants.

Sodium chloride concentrations up to 2 percent was important in the growth of the organism and partly accounted for the results obtained in using the nutrient agar slants.

No appreciable difference in growth was noted between the two strains of the organism that were used, although one had only recently been isolated and the other had been maintained in culture for more than 3 years.

[15] KESSENS, B. H. See footnote 2.

A CRITICAL EVALUATION OF THE RAT-GROWTH METHOD FOR DETERMINING VITAMIN B AND ITS CONTENT IN MEALS FROM CERTAIN OILY SEEDS [1]

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INTRODUCTION

There is little published information on the amount of vitamin B in the more common oil press-cake meals, namely, cottonseed meal, linseed meal, soybean meal, and peanut meal. Morrison (*14, p. 1013*)[2] rates the amount present in peanut meal and in soybean meal as++. Eddy and Dalldorf (*6*) state that cottonseed meal contains 65 International units of vitamin B per ounce. Others (*18, 21*) have reported that cottonseed meal is a good source of this vitamin, but they do not give quantitative figures. Munsell and DeVaney (*15*) found that cottonseed flour contains 5 Sherman units of vitamin B per gram.

The assays reported here were made between 1929 and 1936 by the rat-growth method. In the early work no control rats on a standard of reference were used. Later a sample of dried brewers' yeast was adopted as a standard and fed in parallel with the meals. When the international vitamin B standard became available in 1932 this reference yeast was assayed in terms of International units of vitamin B.

Since the earlier assays could not be evaluated by direct comparison with a standard, it was of interest to determine whether some relation between growth response and vitamin B dosage could be found by which reasonably accurate estimates of the vitamin B potency could be made.

EXPERIMENTAL PROCEDURE

Rats, 22 to 26 days old and weighing 40 to 50 g., were fed vitamin B-free basal ration until they ceased to gain in weight, usually 20 days. They were then placed in individual cages, having raised screen floors, and fed the basal ration and distilled water, ad libitum, together with a definite daily dosage of either the meal being tested or the reference standard, for 7 weeks. The rats were weighed thrice weekly.

The basal ration of Hunt and Krauss (*10*) as modified by Sherwood and Halverson (*17*) was used. It consisted of purified casein (*9, p.122*), 18 percent; washed corn starch, 64 percent; hydrogenated vegetable fat, 10 percent; agar-agar, 2 per cent; cod-liver oil, 2 percent; and McCollum's salt mixture No. 184, 4 percent. The salt mixture was later changed to that proposed by Wesson (*20*), 3.5 percent, and the starch was increased to 64.5 percent. In addition each rat received daily 0.3 g. of autoclaved dried brewers' yeast which had been found to be free from vitamin B.

[1] Received for publication October 11, 1937; issued June, 1938. Read before the Symposium on the Vitamin B Complex, Ninety-third Meeting of the American Chemical Society, Chapel Hill, N. C., April 13, 1937.

[2] Reference is made by number (italic) to Literature Cited, p. 933.

ESTIMATING EQUATIONS

There is no unanimity of opinion as to the form of an equation for expressing the relation between gain response and vitamin B dosage. Supplee, Bender, and Flanigan (*19, p. 373*) state that there is "a direct linear relationship between the rate of growth of white rats and the amount of vitamin B supplied in the ration." Coward, Burn, Ling, and Morgan (*2*) concluded that there is a linear relation between the logarithm of the vitamin B dosage per day and the total gain in the feeding period. Schlutz and Knott (*16*) found that their results indicated a linear equation relating the logarithm of the total vitamin B eaten to the logarithm of the total gain in a given period. Dann and Cowgill (*5*) state that the vitamin B requirement for growth of female rats is proportional to the five-thirds power of the weight.

Preliminary calculations indicated that linear equations of the type

1: International units of vitamin B per day $= a + b$ (total gain) and

2: Logarithm (International units of vitamin B per day) $= a + b$ (total gain)

seemed to fit the data for the rats getting the International standard better than others tried. Equation 1 is similar to that proposed by Supplee, Bender, and Flanigan, and 2 is Coward's equation. The type proposed by Schlutz and Knott did not fit the results very well, the coefficient of correlation for a 4-week feeding period being 0.49.

Table 1 shows the least-square solution of equations of types 1 and 2 for a 4-week feeding period. It also gives the coefficients of correlation and the standard errors for feeding periods of 2 through 7 weeks as computed from the results with the rats receiving the International vitamin B standard.

The results at the end of the first week are not given in table 1 because computation of the coefficients of variation in gain at weekly intervals for different groups of rats at different levels of the International vitamin B standard or of either of four samples of dried brewers' yeast, invariably showed that the variation in gain was greater at the end of the first week than at any subsequent period. After the second (occasionally the third) week there was little change in the coefficient of variation provided the vitamin B dosage was above the maintenance level.

TABLE 1.—*Least-square solution of equations of types 1 and 2 for a 4-week feeding period, and coefficients of correlation and standard errors for feeding periods of from 2 to 7 weeks computed from results with rats receiving the International vitamin B standard*

Assay period (weeks)	Rats	Correlation coefficients		Standard error	
		Arithmetic	Semilog-arithmic	Arithmetic	Semilog-arithmic
	Number				
2	49	0.75	0.77	0.22	0.112
3	49	.71	.77	.23	.112
4	46	.79	.92	.20	.062
5	44	.80	.82	.20	.101
6	40	.86	.85	.17	.091
7	37	.83	.79	.13	.102

Estimating equations—4 weeks:
1, arithmetic; International units of vitamin B = 0.0148 (gain) + 0.606.
2, semilogarithmic; log. International units of vitamin B = 0.0080 (gain) − 0.2367.

The coefficient of correlation based upon the arithmetic relation between gain and dosage apparently increases with the length of the test period. However, calculation of the significance of the differences between these coefficients by means of Fisher's z transformation (*8, p. 182*) shows that they are not significant but may be due to chance. The coefficients based on the semilogarithmic relation (equation 2) seem to reach a maximum at 4 weeks, but again there is no *significant* difference between them. Both sets of correlation coefficients have about the same order of magnitude. When tested by the analysis of variance method (*8, p. 232*) neither of the equations in table 1 is found to differ significantly from the linear. For these reasons there is little choice between these two types of estimating equations.

LENGTH OF FEEDING PERIOD

Because of the seasonal variation in response (discussed later), the comparatively small number of rats used, and the absence of significant differences between the correlation coefficients for the different time intervals, this analysis does not show definitely the optimum length of test period. It indicates that a 2-week period is about as accurate as a longer one. Coward (*1*) has concluded from her studies on a much larger number of rats that a 2-week feeding period yields more accurate results than a 1-week test and that "the increased accuracy obtained by carrying on a test for longer than two weeks is seldom worth the extra labour involved." Schlutz and Knott (*16*) have found that a 10-day feeding period yields valid results provided certain precautions, which they enumerate, are observed. On the other hand, Lassen (*12*) concludes that a 5-week feeding period yields the smallest mean error.

More or less arbitrarily the arithmetic equation for the 4-week period (equation 1, table 1) was used in estimating the vitamin B content of those meals that were tested before the known standards of reference were adopted. Assuming that environmental and other uncontrolled factors did not vary enough to invalidate the application of this equation to the earlier results, the standard error (0.21) shows that the vitamin B potency calculated from the growth response of a single rat will not vary from the true value by more than ± 0.42 of an International B unit in 95 percent of the trials. In the later assays 10 rats were used on each of at least 2 dosage levels of the meals; but in the earlier work there were usually only 6 replicates. The accuracy of the equation with a probability of 0.05, for six rats, is twice the standard error divided by the square root of $6-1$, or approximately ± 0.2 of an International unit of vitamin B.

VITAMIN B IN OIL PRESS-CAKE MEALS

The results of the vitamin B assays are given in table 2. The sample of cottonseed flour, one sample of cottonseed meal, one of soybean meal, and two of peanut meal were run without reference standards, hence the vitamin B content was computed by means of the estimating equation. The potency of the raw, shelled, Virginia Runner peanuts was calculated from previously published (*17*) growth-response data from this laboratory. Daniel and Munsell (*4*)

TABLE 2.—*International units of vitamin B per gram in press-cake meals and other products*

Product	Samples	International units of vitamin B per gram	
		Mean	Range
	Number		
Soybean meal	4	1.5	1.1–1.7
Soybean meal, solvent process	1	3.9	
Meal from Spanish peanuts	2	2.7	2.6–2.7
Meal from Virginia Runner peanuts	2	2.5	2.4–2.6
Cottonseed meal, 36 percent protein	5	3.8	3.0–5.2
Cottonseed meal, 41 percent protein	1	5.0	
Cottonseed flour (Allison flour)	1	4.4	
Linseed meal	3	4.3	3.3–5.4
Raw Virginia Runner peanuts	1	2.4	
Cottonseed-hull bran	1	0	

have used these same data for computing the vitamin B content of peanuts in terms of Sherman units. The amount of vitamin B in cottonseed-hull bran was too small to be detected when fed at a level of 1 g per day.

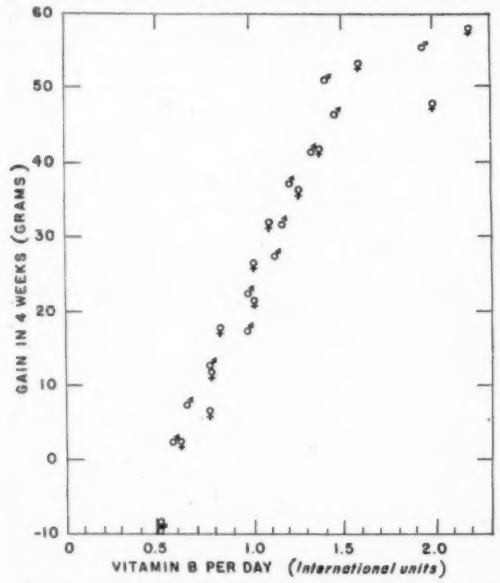

FIGURE 1.—Relation between vitamin B dosage and gain response according to the sex of the test rat.

RELATION OF SEX TO GROWTH RESPONSE

During the course of these assays an impression was obtained that the sex of the rat had little effect on the gain, although approximately equal numbers of males and females were used in each test. In order to clarify this point the average daily vitamin B intake of each rat used in these assays (a total of 670 rats) was computed. These figures were classified according to the sex of the rat and then grouped according to the gain in 4 weeks. The mean gain and the mean vitamin B intake for each group were then determined and plotted, as shown in figure 1.

Figure 1 shows that there is no essential difference in the growth response of the two sexes.

Figure 1 illustrates also the linear relation between total gain and International units of vitamin B per day. The points fall approximately on a straight line except at the ends. The lower end shows

that 0.6 International unit per day is the minimum average amount required for maintenance and that losses in weight are not proportional to the vitamin B intake. Since comparatively few rats received as much as 1.6 International units per day no high degree of confidence can be placed in the extreme upper end of the curve, but the results indicate that the linear relation does not hold for females making gains of 45 g or more in 28 days or for males whose gains are more than 50 g. Apparently the growth response is slightly less for a given increase in vitamin B dosage above 1.5 units than it is when the dosage lies between 0.6 and 1.5 units per day. The fact that Coward's data (*1*) extended considerably beyond these limits may account for her finding a semilogarithmic relation between gain and vitamin B intake.

SEASONAL VARIATION IN GROWTH RESPONSE

General observation, while making the assays, indicated that there is a distinct seasonal variation in response to a given dosage of vitamin B. This variation is relatively unimportant when adequate controls on a known standard are used in parallel with the test animals. However, it does have a bearing on the interpretation of results when the vitamin B unit employed is based upon the growth rate or when a curve of reference is used to evaluate the results, as in the earlier assays reported here.

To determine seasonal variation, a joint-function analysis between the vitamin B dosage of all 670 rats used in the assays and the gain in 28 days and the month in which the assay was started was made by the method described by Ezekiel (*7, pp. 277–294*). The results are summarized in figure 2.

This series of curves shows a distinct seasonal variation in growth response for a given daily dose of vitamin B. From September to February 0.6 of an International unit of vitamin B per day is not sufficient to supply the maintenance requirements of the rats. Beginning in February and continuing to June, this amount of the vitamin promotes increasing gains, but about the first of July the needs of the animals increase so that by the first of September 0.6 of a unit is just sufficient for maintenance of weight. In June 0.6 of a unit promotes a gain of approximately 3 g per week, but in October 0.85 of a unit is required for the same gain. With larger doses of vitamin B the effect of seasonal variation becomes less marked and the peaks and valleys of the curves are shifted somewhat to the left.

Since the data for the individual years varied somewhat from the averages shown, the curves in figure 2 should not be used as curves of estimate. Their use may yield fair approximations of vitamin B potency, but neither these curves nor the estimating equations given above are adequate substitutes for controls on a reference standard, maintained as a part of the assay.

While the seasonal variation in vitamin B requirement does not coincide with the variation in the gains of normal rats as found by Levene (*13*), it is interesting to note that Cowgill (*3, p. 136*) quotes Braddon as saying relative to beriberi in the Singapore Criminal Prison, "* * * an outbreak occurred, as in former years, in August." Also, it is reported (*11, p. 22*) that a condition apparently due to a deficiency of vitamin B becomes common among infants in Palestine with the approach of hot dry winds.

SUMMARY

Soybean meal, peanut meal, cottonseed flour, cottonseed meal, and linseed meal have been found to be good sources of vitamin B. The values ranged from 1.1 International units of vitamin B per gram for a sample of soybean meal to 5.4 International units per gram in one sample of linseed meal. The other meals had intermediate values.

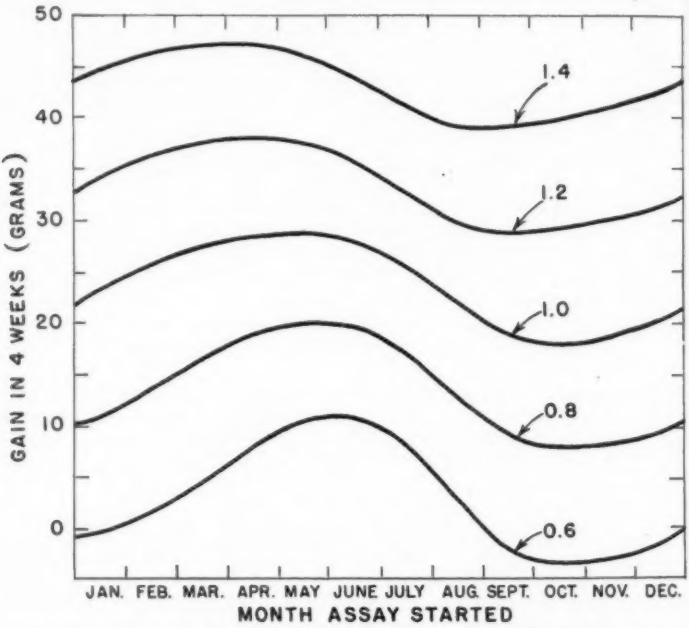

FIGURE 2.—Seasonal variations in gain of rats on different levels of vitamin B. Figures indicate dosage in International units.

Cottonseed-hull bran did not contain an appreciable amount of vitamin B.

Raw shelled peanuts were found to contain 2.4 International units per gram when old data were interpreted by means of the estimating equation given in the text.

A statistical study of the results of the assays by the rat-growth method shows that:

(1) A 7-day feeding period is not long enough for the most accurate results.

(2) There is no advantage in continuing the assay longer than 3 weeks.

(3) The relation between vitamin B dosage per day and total gain in the assay period may be expressed by a linear equation, provided the total gain is less than 50 g in 4 weeks and the daily vitamin B dosage lies between 0.6 and 1.4 International units.

(4) When the vitamin B dosage is within the limits just given there is no difference in response due to the sex of the rat.

(5) There is a seasonal variation in growth response to a given dose of vitamin B. This variation is most marked when the amount of vitamin B is only slightly greater than that required for maintenance. Rats apparently need less vitamin B in the spring and early summer than in late summer and autumn.

(6) A curve of reference may be helpful in interpreting the results of an assay, but it should not be substituted for adequate controls on a reference standard.

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